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Copper oxide nanoparticles and bulk copper oxide, combined with indole-3-acetic acid, alter aluminum, boron, and iron in *Pisum sativum* seeds

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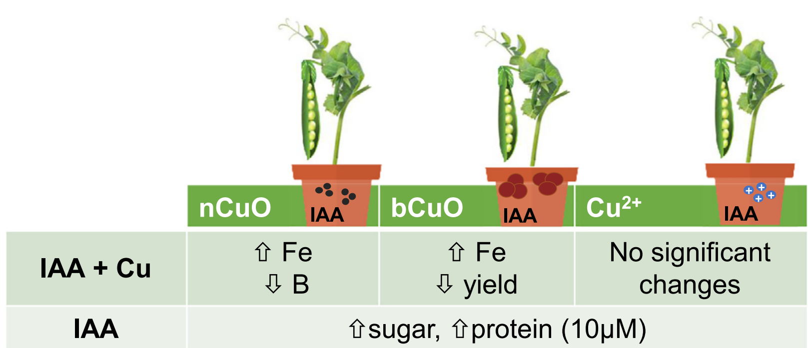
HIGHLIGHTS

- Indole-3-acetic acid (IAA) altered the effects of Cu-based compounds.
- Bulk CuO at 50 mg kg^{-1} reduced seed yield by 58%.
- nCuO at 100 mg kg^{-1}, plus IAA at 100 μM, increased Fe in seeds by 42%.
- nCuO at 50 mg kg^{-1}, plus IAA at 100 μM reduced B in seeds by 80%.
- IAA, at 10 μM increased seed protein by 33%.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction of CuO nanoparticles (nCuO), a potential nanopesticide, with the growth hormone indole-3-acetic acid (IAA) is not well understood. This study aimed to evaluate the nutritional components in seeds of green pea (*Pisum sativum*) cultivated in soil amended with nCuO at 50 or 100 mg kg^{-1}, with/without IAA at 10 or 100 μM. Similar treatments including bulk CuO (bCuO) and $CuCl_2$ were set as controls. Bulk CuO at 50 mg kg^{-1} reduced seed yield (52%), compared with control. Bulk CuO at 50 mg kg^{-1} and nCuO at 100 mg kg^{-1}, plus IAA at 100 μM, increased iron in seeds (41 and 42%, respectively), while nCuO at 50 mg kg^{-1}, plus IAA at 100 μM reduced boron (80%, respect to control and 63%, respect to IAA at 100 μM). IAA, at 10 μM increased seed protein (33%), compared with control ($p \leq 0.05$). At both concentrations IAA increased sugar in seeds (20%). Overall, nCuO, plus IAA at 10 μM, does not affect the production or nutritional quality of green pea seeds.

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1. Introduction

The current production of engineered nanomaterials (ENMs) surpasses 300,000 metric tons/year (Medina-Velo et al., 2017a). Several ENMs, particularly metal oxide nanoparticles, are of interest in various industries because of their electrical (Maruthupandy et al., 2016), magnetic (Joshi et al., 2006; Bonfrate et al., 2017), and catalytic properties (Hattori et al., 2016), among others. Either by accident or intentionally, organisms are exposed to ENMs, raising concerns about the consequences of such exposure (Medina-Velo et al., 2017a). The negative effects and practical applications of metal oxide nanoparticles are being investigated in agriculture production. Dimkpa et al. (2017) found that nanoparticulate ZnO (nZnO) promotes nutrient uptake in sorghum grown under low N, P, and K. In soybean however, nZnO at 500 mg kg^{-1}, reduced Fe in leaves (Peralta-Videa et al., 2014) and inhibited seed production (Yoon et al., 2014). Other studies have shown that $nTiO_2$, at ~90 mg kg^{-1}, reduced wheat biomass (Du et al., 2017), while $nCeO_2$, at 500 mg kg^{-1}, increased barley biomass by >300%, compared with control, although no seeds were produced (Rico et al., 2015).

As the focus of research has expanded from implications to applications, nanoparticles (NPs) are being studied to maximize crop production and/or reduce plant diseases (Servin et al., 2015). Elmer and White (2016) reported that in plants grown in *Fusarium* infested soil, CuO NPs (nCuO) increased tomato and eggplant yield by 33% and 34%, respectively, although no direct disease suppression was observed. Zuverza-Mena et al. (2015) found that nCuO, at 80 mg kg^{-1}, increased shoot biomass and B in cilantro. On the other hand, it was previously noted that nCuO diminished root length and enhanced root lignification in soybean (Nair and Chung, 2014). It also increased lipid peroxidation and reactive oxygen species (ROS) in green pea seedlings (Nair and Chung, 2015), modified the elemental profile, and enzymatic activity in lettuce and alfalfa (Hong et al., 2015).

There is a great volume of information regarding the effects of ENMs in plants (de la Rosa et al., 2017; Du et al., 2017; Zuverza-Mena et al., 2017). Notwithstanding, contradictory results are often encountered and more robust assays in terrestrial systems are needed (Servin and White, 2016). There is a limited volume of data about the response of plants to the exposure of copper ENMs and co-contaminants (De La Torre-Roche et al., 2013; Apodaca et al., 2017; Deng et al., 2017; Ochoa et al., 2017). Phytohormones are growth promoting substances that, very likely, interact with ENMs in plants. One of the best known phytohormones is the indole-3-acetic acid (IAA), an auxin that stimulates plant growth (Simon and Petrášek, 2011). Different species of plants have varying responses to auxin related molecules (Enders and Strader, 2015); thus, the effects of the interaction of ENMs with IAA is expected to vary with the plant species. Studies have shown that the soil composition changes the effects of ENMs in the nutritional quality of bean seeds (Majumdar et al., 2016; Medina-Velo et al., 2017b). Thus, the combination of ENMs with an excess of IAA could affect the physiological and biochemical functions in plants, including changes in the composition of seeds. This manuscript is a follow up of a previous investigation where the physiological and agronomical parameters of green pea (*Pisum sativum*) exposed to nCuO, bulk CuO, and $CuCl_2$, plus indole-3-acetic acid (IAA), were investigated (Ochoa et al., 2017). To the authors' knowledge, there are no reports on the effects of nCuO in green pea seed production and nutritional quality, under IAA exposure. In the current study, data on number of seeds, biomass, crude protein, carbohydrates, bioaccumulation of Cu, and essential elements are discussed.

2. Materials and methods

2.1. Characteristics of nCuO, bCuO, and preparation of suspensions/solutions

Copper oxide NPs (nCuO), bulk CuO (bCuO), and copper(II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$) (Sigma-Aldrich), were obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). According to a previous characterization (Hong et al., 2015), nCuO have a primary particle size of 10–100 nm, 74.3 (wt%) of Cu, hydrodynamic diameter of 280 ± 15 (nm), and a zeta potential (ζ) of −34.4 ± 0.5 (mV). Bulk CuO has a primary particle size of 100–10,000 (nm), 79.7 (wt%) of Cu, hydrodynamic diameter of 376 ± 26 (nm), and a ζ of −42.7 ± 0.2 (mV). Suspensions of nCuO, bCuO, and $CuCl_2$ were prepared at 0, 50, and 100 mg kg^{-1} (in terms of Cu content) in 250 mL volumetric flasks, using Millipore® water (Ochoa et al., 2017). To avoid aggregation, suspensions/solutions were sonicated for 30 min in a water bath at 25 °C (Crest Ultrasonics, Trenton, NJ, Model 275 DA; 120 V, 3 amp, 59/60 Hz). Levels of CuO were selected after Nair and Chung (2014), with a reduced number of concentrations to avoid complexity, due to the number of Cu compounds. In addition, solutions of IAA (Aldrich Chemicals) were prepared at 10 and 100 μM (1.75 and 17.5 mg L^{-1}, respectively), as described by López et al. (2007a). Table 1 shows the factorial experiment [two main factors (copper-based compounds) at three concentrations each and IAA at three concentrations] with the combination of products to generate the 21 treatments used in this study, as previously described (Ochoa et al., 2017).

2.2. Soil type and application of the suspensions

Natural soil (Loam: 19% clay, 44% silt, and 36% sand) was collected from a cotton field in Socorro, TX (N 31° 40.489′, W 106° 17.198′, elevation: 1115 m asl). The soil sampling and classification was previously described by Ochoa et al. (2017) Briefly, samples were taken at a depth from 15 to 60 cm, air dried, and amended with Cu suspensions/solutions to obtain final concentrations of 50 and 100 mg of Cu/kg of soil. The amended samples (2 kg per pot) were thoroughly mixed until homogeneous and kept for 24 h for equilibration. The element concentration in soil and soil characteristics are shown in Table S1. The whole experiment included a total of 84 pots with four replicates per treatment, including an absolute control (no Cu, no IAA) and controls for the IAA and Cu treatments.

2.3. Seed planting and growth conditions

Before planting, the green pea seeds (Little Marvel Dwarf 1454, Ferry Morse, Norton, MA) were surface sterilized and sown as previously described. Pots containing five (Bonfrate et al., 2017) seeds each were randomly set in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) with a 14 h photoperiod, temperature of 25/20 °C day/night, 65–70% relative humidity and light intensity of 340 μmole $m^{-2}s^{-1}$. The planted pots were watered with the respective IAA solution (10 or 100 μM) five days after planting, and in the subsequent five days, seedlings were sprayed with the fungicide (OHP® CHIPCO® 26,019 N/G), following the manufacturer instructions. Plants were watered daily with 50–60 mL of deionized water until full maturity.

2.4. Treatment effects on seed yield

After 90 days of growth, the seeds were harvested, washed with 0.01 M of HNO_3 solution and rinsed three times with Millipore® water

Table 1
Total treatment combinations. The first number indicates the IAA concentration and the second number the Cu concentration.

IAA (μM)	Cu (mg kg^{-1}) of soil Control (0)	nCuO50	nCuO100	bCuO50	bCuO100	$CuCl_2$ 50	$CuCl_2$ 100
0	0, 0	0, 50	0, 100	0, 50	0, 100	0, 50	0, 100
10	10, 0	10, 50	10, 100	10, 50	10, 100	10, 50	10, 100
100	100, 0	100, 50	100, 100	100, 50	100, 100	100, 50	100, 100

to remove any external contaminants. The number of seeds per plant/treatment was recorded. Then, seeds were placed in paper envelopes, and oven dried at 60 °C for 72 h for further analysis.

2.5. Copper and essential element analyses in seeds

Copper and other essential elements were analyzed in samples of 0.2 g of oven dried seeds (72 h at 60 °C). Four mL of NHO_3 were added to the samples (Sigma-Aldrich 67–70%) and placed on a DigiPREP MS digestion block (SCP Science, NY) for 45 min at 115 °C. The digests were diluted with Millipore® water up to 50 mL, analyzed for Cu, Fe, Mn, Zn, Ni, P, K, Ca, Mg, Mo, B, Cl, and S. Aluminum was also analyzed since this non-essential element has shown to be affected by other ENMs (Trujillo-Reyes et al., 2014). All elements were examined in an inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer optima 4300 DV). For quality assurance/quality control, blanks, spikes, and a certified standard reference material for plants (spinach leaves NIST-SRM 1570a) were analyzed, obtaining 98% recovery for Cu, with a detection limit of 1.8 μg L^{-1}. In addition, a multi-elemental standard of 0.5 mg L^{-1} was analyzed every 10 samples to monitor the matrix effect on the analytes and for quality assurance/quality control (Majumdar et al., 2014).

2.6. Crude protein analysis in seeds

Seeds were analyzed for crude protein, regarding total nitrogen content. Dried seeds (72 h at 60 °C) were powdered with mortar and pestle, and sieved in a 650 μm nylon mesh. All samples were 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 (Bremner, 1996; Allen, 2002).

2.7. Total sugar and starch analysis in seeds

Green pea seeds were analyzed for total sugar and starch according to Verma and Dubey (2001), Masuko et al. (2005), and Dubois et al. (1956). Seeds were oven dry at 70 °C for 72 h and pulverized using a mortar and pestle. Later, the samples were homogenized and 100 mg of sample was mixed in 10 mL of 80% ethanol, and boiled in a water bath at 80 °C for 30 min. Subsequently, samples were centrifuged at 22,000 ×g for 20 min. This procedure was repeated three times, and the extracts were placed together. The extract was evaporated to 3 mL and diluted with Millipore® water up to 25 mL. Then, 50 μL of the sample were mixed with 150 μL of sulfuric acid and set in each one of a 96-well microplate. After that, 30 μL of 5% phenol was added, heated for 5 min at 90 °C, and the absorbance measured at 490 nm. The total sugar content was estimated calorimetrically by using phenol sulfuric acid calculations, as per Dubois et al. (1956).

2.8. Statistical analysis

All the analyses were performed in data obtained from four replicates per treatment allocated in a completely randomized design. A one-way ANOVA was carried out to analyze treatments against control. In order to consider the interaction among the two factors (Cu compounds and IAA), a factorial two-way ANOVA was performed. (Statistical Package for the Social Sciences 22, SPSS, Chicago, IL). Analyses were followed by Tukey's multiple comparisons test with a probability of $p \leq 0.05$.

3. Results and discussion

3.1. Seed production

Although none of the treatments affected the seed dry weight (Table 2), some of them significantly reduced the number of seeds. Control plants (no Cu, no IAA) had the highest average number of seeds, which showed a reduction in treatments having IAA. The two-way ANOVA (Table 2) showed a factor effect since bulk CuO, at 50 mg kg^{-1}, without IAA, reduced the average number of seeds by 52%, compared with control ($p \leq 0.05$). However, at 100 mg kg^{-1}, the effect was not larger than for the others, which could be coincidence. In addition, there were interaction effects. Table 2 also shows that IAA at 10 μM, alone, or mixed with nCuO at 50 mg kg^{-1}, with bCuO at 100 mg kg^{-1}, or with $CuCl_2$ at 50 mg kg^{-1}, significantly reduced the average number of seeds (57%, 57%, 54%, and 46%, respectively). Similar results have been found with the higher dose of nCuO and $CuCl_2$. Aminullah et al. (2000) reported that IAA at 10 mg L^{-1} did not affect seed production or seed weight in soybean. Conversely, results have been observed in other plants. In *Streptocarpus nobilis* (C. B. Clarke), a short-day plant, manipulated to induce *in vitro* flowering, IAA inhibited flowering in non-inductive photo-periods (Simmonds, 1987). In *P. sativum* the effect of the auxin 4-Chloroindole-3-acetic Acid (4-Cl-IAA (1×10^{-4} M) on grain yield was null if the plants were exposed to 35 °C during initial flowering (Abeysingha, 2015). Quittenden et al. (2009) indicated that in green pea, tryptophan is a precursor for IAA formation. In addition, Chourey et al. (2010) reported that in the *miniature1* seed mutant of *Zea mays*, there is tryptophan-dependent synthesis of IAA in developing seeds. There is the possibility that the Cu treatments increased the synthesis of tryptophan, which in combination with IAA at 10 μM, affected the seed production. More studies are needed in order to understand the interaction of Cu with IAA in pea plants.

3.2. Copper concentration in seeds

Concentrations of Cu in seeds are shown in Fig. 1. The two-way ANOVA showed no effects of the interaction Cu × IAA (Fig. 1a). Similarly, the main factor analysis showed no effects of the Cu treatments (Fig. 1b). Rawat et al. (2018), reported that the significantly higher release of Cu ions to the soil solution from *n*CuO, compared with *b*CuO, was not reproduced in the Cu content of bell pepper fruits. However, the main factor IAA at 100 μM showed a significant reduction of Cu content in seeds. Very likely, this was produced by the reduction observed

Table 2
Seed production and characteristics of the seeds harvested from plants cultivated for 90 days in soil amended with nCuO, bCuO, and $CuCl_2$ at 50 and 100 mg kg^{-1}, plus IAA at 10 or 100 μM. Average of seed and dry weight per treatment are means ± SE. Averages with the same letter are statically similar ($p \leq 0.05$).

ID	IAA (μM)	Cu compound (mg kg^{-1} d wt)	Total seeds per treatment	Average # of seed per treatment	Avg. seed dry weight per treatment (g)	Dry weight of 10 seeds (g)
1	0	0	58	14.5 ± 1.4a	2.1 ± 0.3abc	1.43
2		n50	36	9.0 ± 0.8ab	1.4 ± 0.2abc	1.58
3		n100	47	11.8 ± 0.8ab	1.8 ± 0.2abc	1.54
4		b50	28	7.0 ± 1.1b*	1.4 ± 0.3abc	2.05
5		b100	45	11.3 ± 1.3ab	2.1 ± 0.2abc	1.89
6		$CuCl_2$ 50	42	10.5 ± 0.3ab	1.8 ± 0.1abc	1.72
7		$CuCl_2$ 100	47	11.8 ± 1.7ab	1.9 ± 0.1abc	1.63
8	10	0	25	6.3 ± 1.3b	1.2 ± 0.1abc	2.00
9		n50	25	6.3 ± 0.9b	1.2 ± 0.0abc	2.00
10		n100	31	7.8 ± 1.1b	1.3 ± 0.3abc	1.67
11		b50	35	8.8 ± 1.9ab	1.6 ± 0.3abc	1.84
12		b100	20	6.7 ± 0.9b	1.1 ± 0.3bc	1.66
13		$CuCl_2$ 50	31	7.8 ± 1.3b	1.5 ± 0.2abc	1.92
14		$CuCl_2$ 100	25	6.3 ± 1.0b	1.1 ± 0.2c	1.71
15	100	0	45	11.3 ± 0.6ab	2.2 ± 0.1abc	1.99
16		n50	33	11.0 ± 1.0ab	2.1 ± 0.1abc	1.94
17		n100	51	12.8 ± 1.0ab	2.3 ± 0.2a	1.79
18		b50	50	12.5 ± 1.7ab	2.0 ± 0.4abc	1.57
19		b100	50	12.5 ± 0.9ab	2.2 ± 0.0abc	1.73
20		$CuCl_2$ 50	47	11.8 ± 1.8ab	2.0 ± 0.3abc	1.69
21		$CuCl_2$ 100	36	12.0 ± 2.1ab	2.3 ± 0.3ab	1.88

with bulk CuO and 10 μM IAA, although the difference was not high enough to be significant in the interaction analysis. Perhaps with higher number of replicates, it would be significant. These results are contrary to the results reported by López et al. (2007b), who found an increase in Pb uptake and translocation in alfalfa exposed to 100 μM IAA and Pb at 40 mg L^{-1}. This might be an effect of plant species or the exposure method. López et al. (2007a, 2007b) performed their experiments in hydroponics and the current study was performed in soil. It has been reported that the majority of Cu stays at root level, since it is one of the most immobile micronutrient in plants (Rusjan, 2012). In addition, Servin et al. (2017), using synchrotron techniques demonstrated that unweathered nCuO remains unaltered (100%) in aggregates of secondary roots and main root, while 31% of the Cu in the epidermis of main root continue to exist as CuO. On the other hand, a previous report indicates that nCuO significantly increased IAA oxidase activity in wheat, which could help the plant to strengthen its resistance to survive (Dimkpa et al., 2012). This suggests that IAA alleviates the stress that Cu compounds might have caused. These results also suggest that the translocation of Cu in pea is independent of the Cu particle size and compound added.

3.3. Protein content in seeds

The data for protein content in seeds is shown in Fig. 2. Similar to the accumulation of Cu in seeds, the analysis of interactions Cu × IAA showed numerical reductions, mainly at 100 μM IAA, but the differences were not high enough to reach statistical significance (Fig. 2a). The main factor analysis showed no effect of the Cu treatments (Fig. 2b); however, the IAA treatments showed an increase in seed protein at 10 μM. A previous report indicates that IAA at low concentration increased protein content in *Catharanthus roseus* G. Don (Muthulakshmi and Pandiyarajan, 2013). In *Arabidopsis thaliana*, the long-term exposure to Cu induced nitric oxide (NO) generation, which has been associated with ROS scavenging and protein nitration (Kolbert et al., 2012; Saxena and Shekhawat, 2013). Thus, the results suggest that the long-term exposure to Cu and the effects of exogenous IAA at low concentration, as well as the interaction of both factors at such concentrations (Gangwar and Singh, 2011), increased the protein nitration in green pea seeds. Lur and Setter (1993) reported that corn (*Zea mays*) kernels defective in IAA had less zeatin, the main protein in corn, compared to wild counterparts. These authors mention that external auxin is related to the number of nuclei in the endosperm. This could result in an increase of protein. However, any conclusions regarding the increase in seed protein under exposure to Cu-based products and IAA would be premature; additional investigation on this topic is needed.

3.4. Element accumulation in seeds

The concentration of elements in seeds that were affected by the treatments are shown in Table 3. As seen in this table, IAA at 100 μM, combined with both nCuO at 100 mg kg^{-1} and bCuO at 50 mg kg^{-1}, increased Fe in seeds by 42% and 41%, respectively, compared with IAA at 100 μM. In addition, IAA at 100 μM, plus nCuO at 50 mg kg^{-1}, increased Al in seeds by 221%, respect to the IAA control, and 297%, compared with the absolute control (no IAA, no Cu added). Conversely, IAA at 100 μM, plus nCuO at 50 mg kg^{-1}, reduced B in seeds by 80%, compared with the absolute control and by 63%, compared with IAA at 100 μM. The data suggests that IAA interacts with particulate Cu, independently of the particle size, interfering with the accumulation of Al, B, and Fe. Unfortunately, to the authors' knowledge, there are no references describing the effects of IAA on the accumulation of elements in seeds. However, IAA has shown to alter the element homeostasis in other plant tissues. For example, the concentration of B and Fe in green pea seeds was similar and contrary, respectively, to the concentration of such elements in leaves of transgenic cotton (Le Van et al., 2016). The increase of Fe in seeds under nCuO or bCuO, plus IAA, could be related to the linkage of the Cu- and Fe-chelate reductase activities in the plasma membrane (Puig et al., 2007). This suggests that CuO was transported from the roots to the leaves, as mentioned by Wang et al. (2012). Subsequently, these particles released Cu(I) and Cu(II) ions (Wang et al., 2012), which interfered with the reductase activity. Concerning B, Singh et al. (1990) reported an inverse relationship between B and Cu

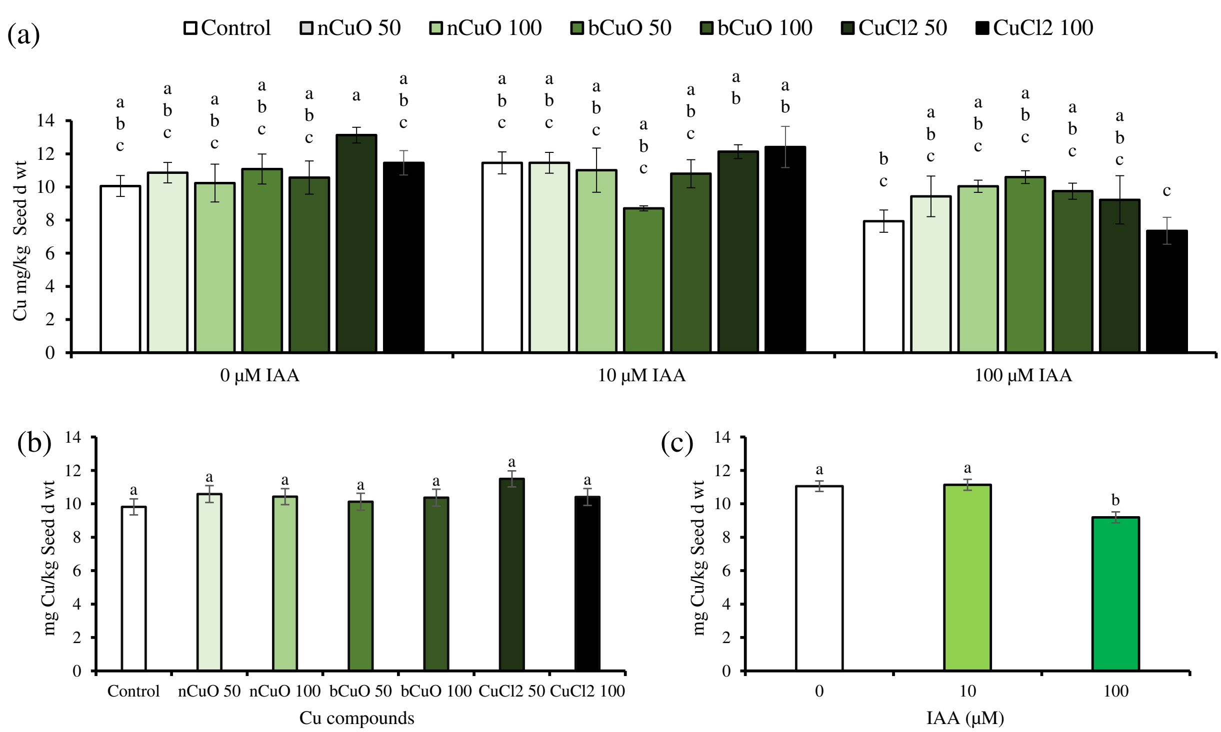

Fig. 1. (a) Copper concentration in green pea seeds from plants cultivated for 90 days in natural soil amended with nCuO, bCuO, and $CuCl_2$ at 50 or 100 mg kg^{-1}, plus IAA at 10 or 100 μM. (b) Copper concentration in seeds averaged by the main factor Cu compounds, and (c) by the main factor IAA. Results are means ± SE. The statistical differences are indicated with letters and * indicate differences when compared to the respective control ($p \le 0.05$).

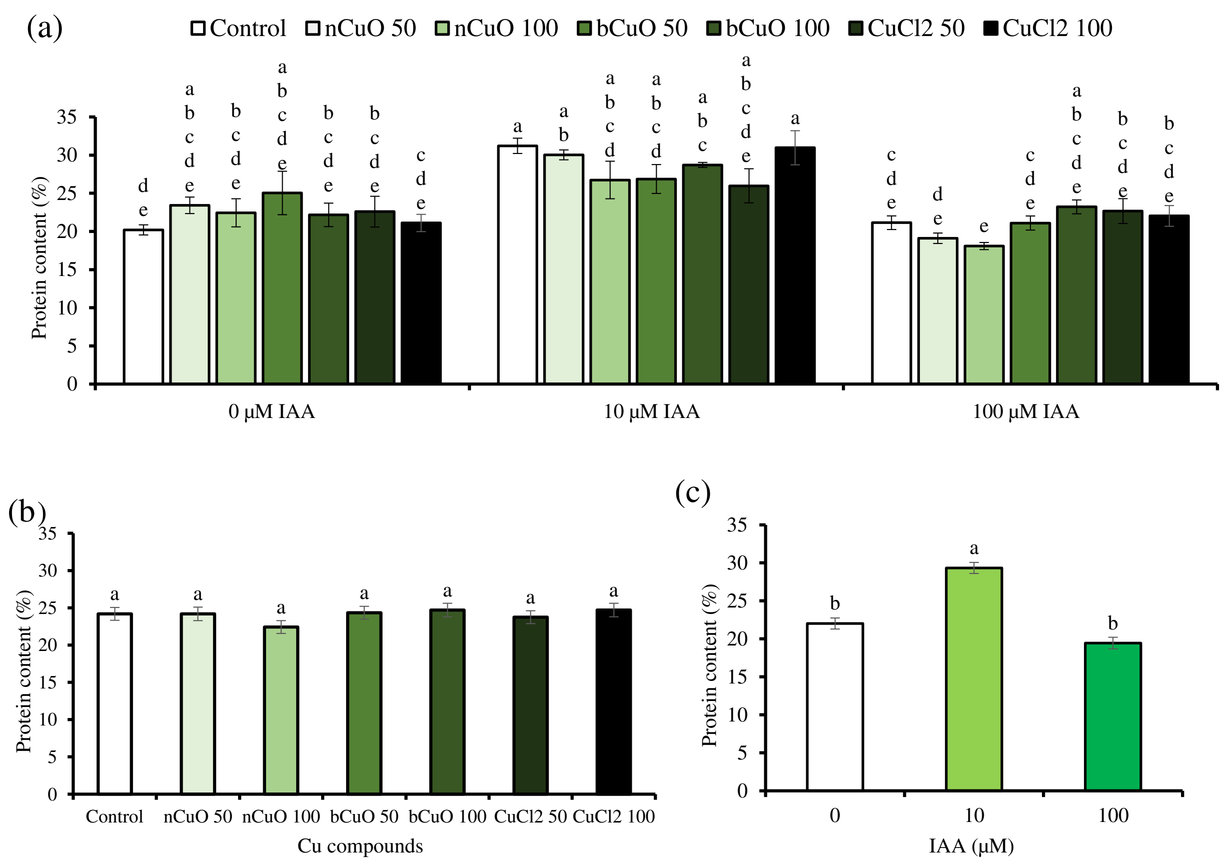

Fig. 2. (a) Protein content (%) in green pea seeds from plants grown for 90 days in natural soil amended with nCuO, bCuO, and $CuCl_2$ at 50 or 100 mg kg^{-1}, plus IAA at 10 or 100 µM. (b) Statistical differences for the number of leaves averaged by the main factor Cu compounds, and (c) grouped by the main factor IAA. Results are means ± SE. The statistical differences are indicated with letters and * indicate differences when compared to the respective control ($p \leq 0.05$).

uptake in wheat, which could also happens in green pea. The results of element accumulation in pea seeds would have a contradictory meaning. On one hand, an increase in Fe would be beneficial, since this element is highly required in humans; on the other hand, an increase in Al could represent a threat, since an excess in Al has been associated with Alzheimer's disease (Tomljenovic, 2010).

3.5. Sugar and starch content

Carbohydrates are the main constituents in legume seeds. In green pea, carbohydrates comprise about 57% of the seed weight, being starch the most abundant (from 37 to 49%) (Bressani and Elias, 1988). Our results showed that the combination of Cu-based compounds with IAA had no effect on carbohydrates of pea seeds (Figs. 3 and 4). However, the main factor IAA, at both concentrations, increased sugar in seeds by about 20% (Fig. 3c), while at 100 µM, it increased starch by 29% (Fig. 4c). According to Locascio et al. (2014), auxins have a fundamental role in seed development, particularly in endosperm, which contain starch and sugar. An excess in auxin in seeds promotes cellular differentiation in endosperm, which in turn, resulted in an increase in sugar and starch (Locascio et al., 2014).

4. Conclusions

Results of this study suggest that nCuO, at the concentrations tested, did not affect green pea production; however, in absence of IAA, bCuO at 50 mg kg^{-1}, decreased it by about 50%. The data also suggests that under an excess of IAA, the Cu treatments reduce the number and weight of seeds. There were no significant differences in the Cu translocation from plant organs to seeds. However, the addition of IAA lowered Cu accumulation in seeds by 17%, compared to controls. On the other hand, the main factor IAA, at 10 µM, increased the protein content in

Table 3
Elements affected in green pea seeds from plants grown for 90 days in natural soil amended with nCuO, bCuO, and $CuCl_2$ at 50 or 100 mg kg^{-1}, plus IAA at 10 or 100 µM. Data are average ± SE of four replicates. Comparisons were made between all treatments against absolute control (No Cu, no IAA) and the symbol * represents differences against control 3 (100 µM IAA).

Elements	Treatment	Concentration (mg kg^{-1} d wt tissue)	% change related to absolute Control	% change related to Control 3
Fe	Control (0 µM IAA)	62.8 ± 3.1ab		
	Control 3 (100 µM IAA)	44.0 ± 3.0b		100%
	100 µM IAA + nCuO 100 mg kg^{-1}	62.3 ± 1.6ab*		+42%
	100 µM IAA + bCuO 50 mg kg^{-1}	61.9 ± 3.4ab*		+41%
B	Control (0 µM IAA)	7.1 ± 0.3ab	100%	
	Control 3 (100 µM IAA)	3.8 ± 0.4bcdefg		100%
	100 µM IAA + nCuO 50 mg kg^{-1}	1.4 ± 0.4g*	−80%	−63%
Al	Control (0 µM IAA)	3.4 ± 0.2b	100%	
	Control 3 (100 µM IAA)	4.2 ± 0.3b		+100%
	100 µM IAA + nCuO 50 mg kg^{-1}	13.5 ± 4.1a*	+297%	+221%

Letters indicate statistical differences between all treatments ($p \leq 0.05$).

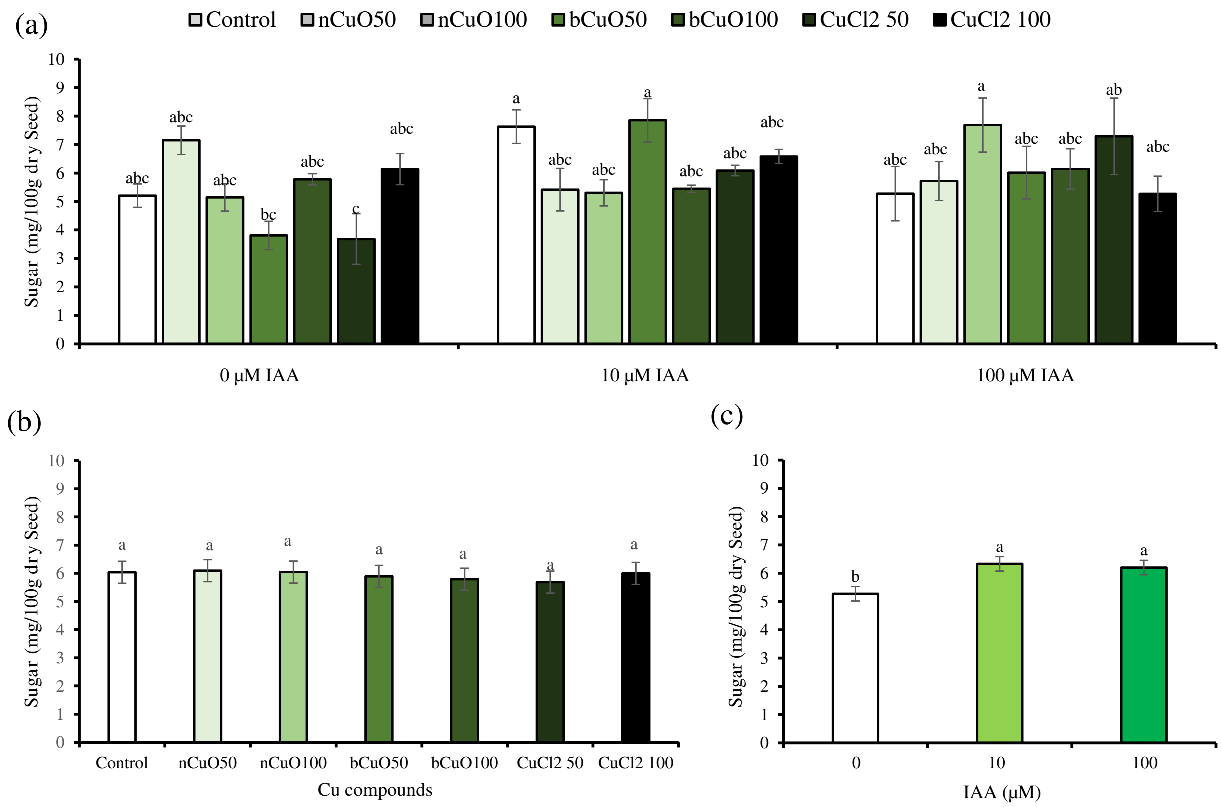

Fig. 3. (a) Total sugar content in green pea seeds from plants grown for 90 days in natural soil amended with nCuO, bCuO, and $CuCl_2$ at 50 or 100 mg kg^{-1}, plus IAA at 10 or 100 μM. (b) Statistical differences on total sugar content in seeds grouped by Cu compounds, and (c) grouped by the main factor IAA. Results are means ± SE. The statistical differences are indicated with letters and * indicate differences when compared to the respective control ($p \le 0.05$).

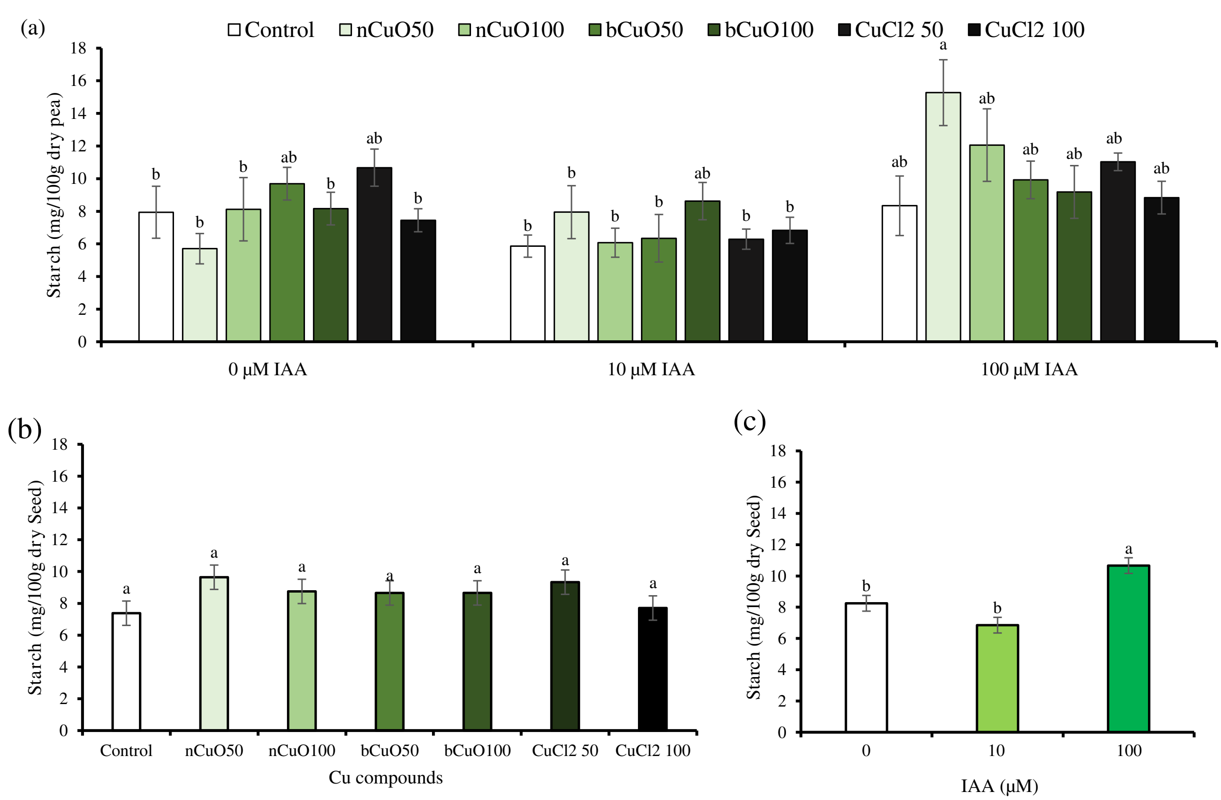

Fig. 4. (a) Starch content in green pea seeds from plants grown for 90 days in natural soil amended with nCuO, bCuO, and $CuCl_2$ at 50 or 100 mg kg^{-1}, plus IAA at 10 or 100 μM. (b) Statistical differences on starch content in seeds grouped by Cu compounds, and (c) grouped by the main factor IAA. Results are means ± SE. The statistical differences are indicated with letters and * indicate differences when compared to the respective control ($p \le 0.05$).

seeds. Although at both concentrations, IAA increased sugar in seeds, the interaction with the Cu-based compounds was not significant. The combination of IAA at 100 μM with the Cu-based compounds altered the concentration of the essential elements Fe and B and the concentration of Al, a non-essential element. Overall, the interaction of nCuO, a nanopesticide, with 100 μM of the phytohorme IAA, seems to reduce the nutritional quality of green pea seeds. However, more studies with different concentrations of IAA and nCuO are needed in order to better understand the effects of the combination of these factors in green production.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.04.003.

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Conflict of interest

The authors declare no conflict of interest.

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