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Evaluating The Effects Of The Phytohormone Indole-3-Acetic Acid In The Response Of Green Pea (Pisum Sativum L.) To Nanoscale CuO Exposure In Soil

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Abstract

The response of plants to copper oxide nanoparticles (*n*CuO) in presence of the phytohormones such as IAA, is unknown. In this study, green pea (*Pisum sativum*) plants were cultivated to full maturity in soil amended with *n*CuO, CuO bulk (*b*CuO), and $CuCl_2$ at 50 and 100 mg/kg and indole-3-acetic acid (IAA) at 10 and 100 µM. Several analytical techniques were used to evaluate the effects of treatments in the agronomical, physiological, and biochemical effects of treatments. Results showed that only $CuCl_2$ reduced root length and number of leaves. Root copper (Cu) increased in plants exposed to *n*CuO without IAA; however, the three Cu compounds, at the highest concentration, increased it when combined with 100 µM IAA. There was a variety of responses in essential nutrient accumulation in plant systems. Except for the combination of nCuO and IAA, both at the highest concentration, nCuO did not affect Ca accumulation in tissues. Different responses were found with the other treatments. Manganese (Mn) decreased by 19% in stems of *n*CuO and $CuCl_2$ treated plants, in the presence of IAA at 10 µM. The highest concentration of nCuO and IAA reduced Mg in stems by 35%. On the other hand, in the absence of IAA, $CuCl_2$ at 50 and 100 mg/kg increased Sulfur (S) in leaves by 217% and 264% and by 93% and 118% in stems, respectively ($p \leq 0.05$). Both the number of seeds and seed weight were reduced in treatments that included IAA at 10 µM. However, the protein content (%) was enhanced in plants treated with 10 µM of IAA. Chlorophyll was affected by $CuCl_2$ at both IAA concentrations, but mainly in treatments involving 10 µM of IAA. Catalase (CAT) activity in roots was reduced in plants exposed to nCuO and 10 and 100 µM of IAA, but increased in treatments with no IAA. Overall, results have shown that the combination of nCuO and IAA caused different effects in pea plants. Unexpectedly, exposure of IAA at 10 µM, and nCuO at 50, resulted in an increase in seed protein compared with the absolute control, which suggests a possible benefit for human nutrition.

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Chapter 1: Introduction

NANOPARTICLES

The use of different nanomaterials (NMs) has increased due to their wide range of applications. The miniature size of NMs (up to 100 nm in at least one dimension) provides for their unique physicochemical characteristics. Nanoparticles (NPs), a special member of the NMs family with dimensions < 100 nm in at least two dimensions (ASTM 2012), have been engineered (ENPs) for several applications, including the improvement of features such as electrical conductivity, physical strength, chemical reactivity, magnetism, among others (Klaine et al. 2008; Peralta-Videa et al. 2011; Keller et al. 2013). The use of NPs in different fields such as cosmetics, pharmaceutical, water and wastewater treatment, agriculture, and food packaging has increased over time (Gottschalk and Nowack 2011; Peralta-Videa et al. 2011; Dimkpa et al. 2013). Along with the use of NMs, concerns emerged about the impacts they may cause in the environment. The ample demand of NPs has raised their industrial production; in 2010 production reached 260,000-309,000 metric tons worldwide, 8-28% of which ended in the soil, 0.4-7% in bodies of water, and 0.1-1.5% in the atmosphere (Keller et al. 2013).

According to the Environmental Protection Agency Nanotechnology White Paper (EPA 2007), bio-solids from wastewater treatment plants end up in agricultural fields. In addition, reports indicate that bio-solids are unintentionally contaminated with ENPs (Gottschalk and Nowack 2011; Keller et al. 2013; Hou et al. 2013), suggesting that field crops could be exposed to them. The resultant, food contamination is a possible threat to human and animal consumers alike. As the use of ENPs increases due to their ample application and wide demand, the chances for contamination of irrigation water and agricultural lands will also increase. These observations

suggests that more studies related to the risk assessment of effects of ENPs in crop plants are necessary.

Use of copper oxide Nanoparticles and other copper products

Copper (Cu) is a transition metal that is found as a pure metal in Earth, and was one of the first metals used by humankind. Copper is one of the essential elements for living organisms including animals and plants. It is used by animals in functions such as respiration, blood production, muscle movement, liver functions, bone formation, and by plants in cell division, and catalysis of redox reactions, just to mention a few (Asada et al., 1977; Fernandes and Henriques 1991; Yruela 2005). At low concentrations, Cu is an essential nutrient for plants. Plant biomass contains it at an average of 10 $\mu g \cdot g^{-1}$ dry weight (Yruela 2005), and is thus considered a micronutrient. This micronutrient benefits plants by promoting the translocation of sugars and liquids, cell metabolism, chlorophyll formation, photosynthesis and lignin synthesis (Fernandes and Henriques 1991; Lopez Torres 2011; copperalliance.org.uk). A deficiency or an excess of Cu, however, can induce irregularities in plant development and growth.

Copper-based products have been widely used in industry, science, and technology. This includes gas sensors, catalysts, electronics, film application, superconductors, agriculture, and in heat transfer nanofluids (Zhu et al. 2004; Nasibulin et al. 2001; Blinova et al. 2010). In agricultural production, it has been used for more than a century as a fungicide and bactericide. Average Cu concentrations in soil vary from 20-30 mg/kg but near copper mining areas, it can reach up to 2000 mg/kg (Nriagu 1979; Freedman and Hutchison 1980; Humphreys and Nicholls 1984; Fernandes and Henriques 1991).

In several experiments Cu has demonstrated to have toxic effects when used in large quantities. Reports mention reductions in shoot and root growth, chlorophyll content, and alteration of stress enzyme activities, just to mention a few (Shah and Belozerova, 2009; Zhenyu et al. 2012; Dimkpa et al. 2012; Nair and Chung 2014). These effects could certainly impact the commercial production of crop plants. On the other hand, products such as $Cu(OH)_2$ have been used for centuries in agriculture to combat fungal diseases in plants (Rusjan 2012). Recent studies have also shown that nanoparticulate copper materials are more effective in controlling some specific plant pathogens such as *Alternaria alternata*, *Botrytis cinerea,* and *Corticium salmonicolor* (Ouda 2014; Du Cao et al. 2014). These beneficial usage suggest that common fungicides and pesticides could be replaced by fungicides and pesticides made with ENPs.

AUXINS (INDOLE-3-ACETIC ACID)

In nature, plants produce growth regulators (PGRs), also known as phytohormones. Phytohormones are naturally produced by plants and can also be synthetically produced. At low concentrations, phytohormones control plant growth in terms of root and stem enlargement, fruit set and drop, and many more developmental processes (Weaver 1982; Lopez Torres 2011; Barbafieri et al. 2012). The main three groups of phytohormones include auxins, gibberellins, and cytokinins, although there are other PGRs that impact specific functions in plant systems (Barbafieri et al. 2012). Synthetic PGRs are used in agriculture to enhance plant growth, development, and crop production.

Auxins are one of the most studied PGRs, well known for their multifunctional roles in plant development and growth. One of the original applications of an auxins was as a weed killer: 2,4-dichlorophenozyacetic acid (2, 4-D). Illustrating how the same phytohormone may well have

different effects on different plant organ, 2, 4-D now also is widely used in agriculture to produce roots in cuttings and transplants (Barbafieri et al. 2012). Indole-3-acetic acid (IAA) is one of the most studied auxins. IAA induces apical dominance, stem elongation, tropisms, and cell division/plant growth, among others (López et al. 2007b; Barbafieri et al. 2012). It also induces the activation of ATPases in the plasma membrane, enzymes that convert ATP to ADP. This activation produces changes in the transportation of ions through the membrane, affecting the accumulation of some macro and micronutrients (López et al. 2005). Liphadzi et al. (2006) reported that sunflowers (*Helianthus annuus*) cultivated in soil containing metals from sewage sludge or composted soil. When these plants were treated with IAA as a spray or added to the soil in combination with ethylenediaminetetraacetic acid (EDTA), more Cd and Pb than controls. López et al. (2007a) showed that in alfalfa (*Medicago sativa*) exposed to Pb, IAA, and EDTA significantly increased the concentration of Cu in roots and Pb in leaves, suggesting modifications in metal uptake and translocation. However, Barbafieri et al. (2012) mentioned that the response of plants to PGRs depends on the application time, weather conditions, stress, and genotypic differences. They therefore suggest that the effect on plants of PGRs and NPs will vary with environmental conditions. All previous reports, however indicate that the growth promotion produced by IAA increases metal uptake. By extension, similar results could occur if the metals were in nanoscale forms.

GREEN PEA PLANTS (*PISUM SATIVUMI* FABACEAE)

Legumes are generally consumed around the world as raw frozen, or cooked. Green peas (*Pisum sativum*), are legumes (Family Fabaceae) rich in essential amino acids (lysine and leucine), protein, vitamins, and such minerals, as sodium, potassium, phosphorus, calcium,

iron, zinc, manganese, magnesium, and copper (Iqbal et al. 2006; Mukherjee et al. 2013). The scientific classification of green pea is shown in **Table 1**.

Due to its easy cultivation, pea plants have been used as model plants for several studies. Famously, Gregor Mendel developed his Mendelian principles of genetics based on plant breeding experiments performed with pea plants. Peas are still widely cultivated worldwide for purposes of engineering, scientific, medical research, as well as human consumption to fulfill a healthy diet. Although pea plants have a low demand for care, there are still some threats that could be devastating to pea crop production. Field grown peas are more susceptible to stress than greenhouse-grown individuals, and subject to a variety of bacterial, viral, and fungal pathogens. The use of fungicides, bactericides, and insecticides is therefore a common agricultural practice for this crop. A common element used in fungicides is copper (Cu). The use of agricultural fungicides necessarily increases the Cu availability in soil. Leading to possible excess of plant uptake of this element.

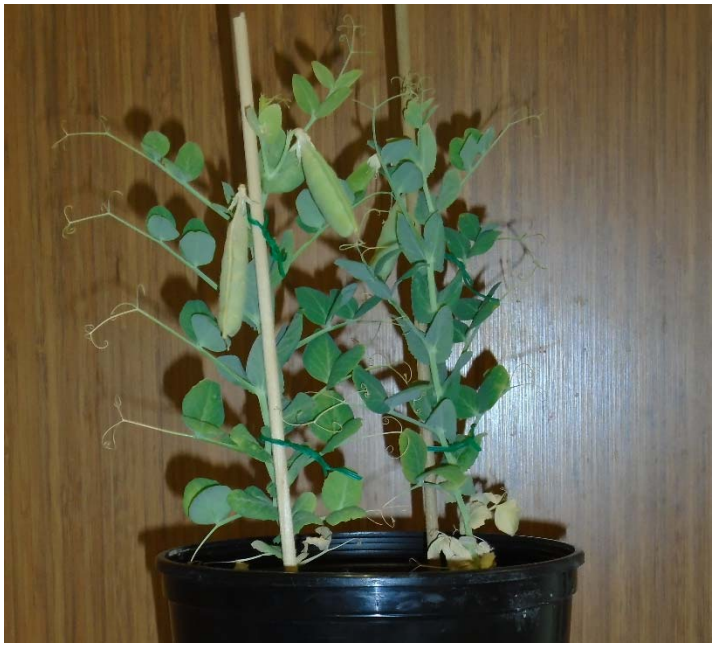

Figure 1 Green pea (*Pisum sativum*)

Table 1 Scientific classification of green pea plant

Domain	Eukarya
Kingdom	Plantae
Phylum	Anthophyta
Class	Eudiotyledones
Order	Fabales
Family	Fabaceae
Genus	*Pisum*
Species	*P. sativum*

RESEARCH OBJECTIVES

The general objectives of this investigation were to investigate the effects of *n*CuO, in combination with IAA in green peas, throughout the life cycle of the plants.

Specific objectives:

1. Determine the effects of the IAA and *n*CuO interaction on the uptake of Cu and other nutrients.

2. Evaluate the effects of treatments on physiological parameters.

3. Evaluate the effects of treatments on agronomic parameters and seed nutritional quality.

Hypothesis

The working hypothesis of this research was that IAA modifies the effects of the *n*CuO or compounds on green pea plants, including growth, production, and seed quality.

Chapter 2: Evaluating the interaction of the phytohormone indole-3-acetic acid in the response of soil grown green pea (*Pisum sativum* Fabaceae) plants to CuO nanoparticles exposure

INTRODUCTION

Previous studies have shown that *n*CuO, at different concentrations, alters plant growth and development. For instance, Dimkpa et al. (2012) reported that these NPs (500 mg Cu/kg sand substrate) decreased shoot growth, shoot chlorophyll content, and oxidized glutathione in 14-day old wheat plants (*Triticum aestivum*). Zhenyu et al. (2012) exposed corn (*Zea mays*) seedlings for 15 days in hydroponics to 100 mg/L *n*CuO and reported a reduction in root length and leaf surface area. In hydroponically grown lettuce (*Lactuca sativa*), *n*CuO at 20 mg/L reduced water content, root length, and dry biomass (Trujillo-Reyes et al. 2014). According to Nair and Chung (2014), soybeans (*Glycine max*) exposed to *n*CuO had a reduction in shoot growth, plant weight, and chlorophyll content. Additionally, Hong et al. (2015) reported that *n*CuO reduced the root length in alfalfa (*Medicago sativa*) and lettuce. In a recent review, Du et al. (2016) highlighted that *n*CuO affects the growth, reactive oxygen species (ROS) production, nutrient uptake and yield characteristics in several plant species.

Different from vertebrate animals, plants do not have a central nervous system to communicate within their different organs and to responds to external stimuli; these control tasks are performed by different types of endogenous hormones (Gaspar et al. 1996; Barbafieri et al. 2012). One of the most studied phytohormones are auxins, more specifically, indole-3-acetic acid (IAA). At the correct amount, IAA promotes apical dominance, stem elongation, tropism, adventitious root formation, and cell division, and other aspects of plant growth (López et al.

2007b; Barbafieri et al. 2012). Zhao (2010) mentioned that an increment in IAA may create a modification of plant development. IAA is known to be used in agriculture to enhance crop growth and roots in cuttings (Barbafieri et al. 2012). A previous study by López et al. (2007a), showed that IAA significantly increased lead (Pb) uptake and translocation in hydroponically grown alfalfa, exposed to 40 mg/L Pb plus ethylenediaminetetraacetic acid (EDTA). Piotrowska-Niczyporuk et al. (2012) reported that in the unicellular green algae *Chorella vulgaris,* when exposed to phytohormones and heavy metals, carotenoids, chlorophyll, protein, ascorbate, glutathione, superoxide dismutase, and catalase increased. These findings suggest that the application of phytohormones can mitigate stress symptoms and the phytotoxicity of heavy metals (Dimkpa et al. 2008; Choudhary et al. 2010; Piotrowska-Niczyporuk et al. 2012).

According to the Environmental Protection Agency (EPA) nanotechnology white paper (2007), bio-solids containing NPs from wastewater treatments have ended up in agricultural fields. Such biosolids increase the chances of crop plants to become exposed to unknown amounts of NPs. The green pea (*Pisum sativum* L.), a legume rich in protein, aminoacids, vitamins, and minerals (Iqbal et al. 2006) could thus be unintentionally exposed to NPs during cultivation. Moreover, bacteria isolated from pea plant root nodules have shown to produce IAA (Tariq et al. 2014). Therefore green pea plants in agricultural situations could become exposed to a combination of IAA and *n*CuO with unknown consequences.

The objectives of the present study were to determine the effects of *n*CuO and IAA on the agronomical, physiological, and biochemical parameters in pea plants. Several treatment techniques were used to evaluate effects on germination, biomass, chlorophyll, carotenoids,

crude protein, enzymatic activity, and bioaccumulation of Cu and other essential elements in tissue.

MATERIALS AND METHODS

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

Copper oxide NPs (*n*CuO), bulk CuO (*b*CuO), 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 previous characterization (Hong et al. 2015), *n*CuO have a primary particle size of 10-100 nm, a Cu content of 74.3 (wt. %), hydrodynamic diameter of 280 ± 15 (nm), and a ζ- potential of -34.4 ± 0.5 (mV). *b*CuO has a primary particle size of 100-10,000 (nm), a Cu content of 79.7 (wt. %), hydrodynamic diameter of 376 ± 26 (nm), and a ζ- potential of -42.7 ± 0.153 ± (mV). Suspensions of *n*CuO, *b*CuO, and $CuCl_2$ were prepared at 0, 50, and 100 mg/kg (in terms of Cu content) in 250 mL volumetric flasks, using Millipore water. To avoid aggregation, suspensions/solutions were sonicated for 30 minutes in a water bath at 25 °C (Crest Ultrasonics, Trenton, NJ Model 275 DA; 120 volt, 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 0, 10, and 100 μM, as described by Lopez et al. (2007a). **Table 2** shows product combinations to generate the 21 treatments used in this study.

Table 2 Total treatment combinations (first number indicates the IAA concentration and the second number the Cu concentration).

	Cu mg/kg of soil						
IAA	Control	*n*CuO50	*n*CuO100	*b*CuO50	*b*CuO100	$CuCl_2$	$CuCl_2$

(μM)	(0)					50	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

Soil collection and application of the suspensions

Natural soil was collected from Socorro, TX (N 31° 40.489', W 106° 17.198', elevation: 1,115 m asl). To avoid root debris and residues of fertilizers, the top portion (first ~15 cm) was removed and the samples were taken from a depth of 15-60 cm (http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs144p2_051273.pdf). The soil was then air-dried and sieved through a 6 mm mesh prior to experimental use. The soil had 19 % clay, 44% silt, and 36% sand, and thus could be classified as loam, (one of the best agricultural soils, according to USDA soil texture classification). Other soil sample properties include pH of 7.825 ± 0.021, electrical conductivity (EC) of 1705 ± 47.6 μS/cm, and total dissolved solids (TDS) of 847.5 ± 23.8 mg/L (Hanna Instruments HI 9813-6 Portable pH/EC/TDS/Temperature Meter). The elemental analysis of the soil is shown in **Table S1**.

A total of 84 general purpose garden plastic pots were previously washed with soap, bleach, and water, dried and rinsed with a mild solution of 5% nitric acid (HNO_3) to remove any contaminants. Each air-dried clean pots were filled with 2 kg of natural soil. The soil was then amended with the Cu suspensions/solutions, manually mixed until getting a homogenous mixture, and kept for 24 h for stabilization. As shown in **Table 2**, there were controls for the IAA and Cu treatments. Four replicate/treatment including control (no NPs, no IAA) were established and after 45 days (beginning of blossom), and 90 days (full maturity), plats were

harvested to evaluate physiological/agronomical parameters such as dry biomass, chlorophyll, carotenoids, and plant elongation, among others.

Seed planting and growth conditions

Green pea seeds (Little Marvel Dwarf 1454, Ferry Morse, Norton, MA) were soaked in a 2% sodium hypochlorite solution for five minutes, rinsed five times with deionized water for surface sterilization, and immersed in Millipore water for 24 h to hydrate. Subsequently, five seeds were planted (~2.5 cm deep) in each pot. Pots were set under controlled conditions in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) with 14 h photoperiod, 25/20 °C day/night, 65-70 relative humidity and light intensity of 340 μmole $m^{-2}s^{-1}$. Five days after planting, the pots were added with the respective IAA solution. To avoid root rot, in the subsequent five days, the fungicide (OHP® CHIPCO® 26019 N/G) was applied as a drench, following the recommendations of the manufacturer. All plants were watered daily with 50- 60 mL of deionized water. No additional fertilizer or nutrient solution was added.

Treatment effects on physiological/agronomical parameters

After 45 days of growth, the plants were harvested and separated in leaves, stems, and roots. The plants were then washed with 0.01 M of HNO_3 solution and rinsed three times with Millipore water, to remove any external contaminants on the surface of the tissue. All plants were counted to record the plant survival at 45 days. Plant organs where counted and measured to evaluate any physiological difference within treatments. After separating and counting plant organs, they were placed in paper envelopes and oven dried at 60 °C for 72 h for further analysis. Similar procedure was followed for the 90 day plants.

Determination of Cu and other nutritional elements in dry plant tissues

The dried samples of plant organs were digested with 4 mL of NHO_3 (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 micronutrients B, Cu, Fe, Mo, Mn, Ni, and Zn, and macronutrients Ca, K, Mg, P, and S by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer optima 4300 DV). *Aqua-regia* digested soil samples were analyzed for the same elements determined in plants. For quality assurance/quality control, blanks, spikes, and certified standard reference material for plant (spinach leaves NIST-SRM 1570a) and soil (NIST-SRM 2709a San Joaquin Soil) were analyzed, obtaining 98% recovery for Cu, with a detection limit of 1.8 µg/L. In addition, 0.5 mg/L multi-elemental standard was analyzed every 10 samples to monitor the matrix effect on the analytes and for quality assurance/quality control (Majumdar et al., 2015).

Carotenoids and Chlorophyll assays

Carotenoids, chlorophyll *a* and *b*, and total chlorophyll contents were determined in 45 day-old pea plants, following the Lichtenthaler and Welburn (1983) method. A sample of 0.1 g of razor blade chopped fresh green pea leaf tissue was grinded with pestle for 5 min in a tissue grinder (Kimble Chase, 5 ml) with 5 mL of 80 % acetone. The extract was kept at – 80 °C until assays were performed. The measurements were performed using a Perkin Elmer Lambda 14 UV/Vis spectrometer at 470, 663, and 646 nm, respectively.

Catalase (CAT) analysis

Catalase (E.C. 1.11.1.6), the main H_2O_2 decomposer enzyme at peroxisome level (Scandalios, 2005), was determined in roots of 45-day old pea plants. The analysis of the catalase (CAT) activity was performed as reported by Gallego et al. (1996). For each replicate/treatment, a sample of 0.2 g of fresh tissue was finely chopped with a razor blade, grinded with mortar and pestle, and extracted with 1800 µL of phosphate buffer solution (25 mM KH_2PO_4, pH 7.4). Extracts were placed in 2 mL Eppendorf tubes and centrifuged for 10 min at -4 °C and at 9600 rpm (Eppendorf AG bench centrifuge 5417 R, Hamburg, Germany). The supernatants were transferred to clean Eppendorf tubes for the enzymatic analysis. An aliquot of 50 µL of each supernatant was transferred to a 1 mL quartz cuvette, added 950 µL of 10 mM H_2O_2, and shaken three times by hand. Absorbance was read for 3 min at 240 nm in a Perkin Elmer Lambda 14 UV/Vis Spectrometer (single-beam mode, Perkin Elmer, Uberlingen, Germany). Data was collected from four replicate/treatments.

Statistical analysis

The treatments were allocated in a completely random design in the growth chamber. For all grouped comparisons against control, the data was analyzed using one-way ANOVA followed by Tukey's multiple comparisons test with a probability of $p \leq 0.05$ (Statistical Package for the Social Sciences 22, SPSS, Chicago, IL). A factorial two-way ANOVA was performed to examine the interaction between the two factors: three copper compounds (three concentrations: 0, 50 and 100 mg/kg soil) and IAA (three concentrations 0, 10, and 100 µM). The Tukey HSD test was used to determine statistically significance difference between treatments means at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Seed germination and plant growth

Table 3 shows the effects of treatments on the average number of plants surviving at 45 days. As seen in **Table 3**, although at 10 and 100 μM IAA, some Cu treatments/concentrations reduced the number of plants; however, the differences were not statistically significant. The average of the main factor, Cu compounds, over all IAA concentrations, showed a reduction with *n*CuO and $CuCl_2$ treatments, but the difference was only numerical (data in appendix **Figure S1b**). On the other hand, averages of the other main factor, IAA, showed a statistical reduction of 23% at 10 μM and 34% 100 μM. Previous reports have shown that heavy metals like Cr and IAA at 100 μM decreased pea seed germination rate, compared with control (Gangwar and Singh, 2001). It is possible that similar interaction occurred with Cu ions/nCuO when interacting with IAA. In consequence, survival of the plant will begin with germination and consecutively affect survival.

The average number of leaves/treatment is shown in **Table 3**. As seen in **Table 3**, the absence of IAA or IAA at 10 μM, did not affect the number of leaves. A comparison between treatments showed that 100 μM IAA+ $CuCl_2$ 50 mg/kg reduced the number of leaves, compared to 100 μM IAA plus bCuO at both 50 and 100 mg/kg. The other treatments resulted in numerical increments and decrements (**Figure S2, apendix**). Naeem et al. (2004) reported that in lentil (*Lens culinaris*), a close relative of green pea, IAA at 0.14 mM (140 μM) increased the number of leaves. This suggests that an excess of IAA in roots counteract the possible oxidation of IAA by the Cu ions/particles (Chaoui et al. 2004).

Table 3 also shows that none of the treatments affected the shoot system length. However, some significant differences were recorded in stems and roots. Treatments with no IAA and with nCuO, at both concentrations, and bCuO at 50 mg/kg, increased roots length, while $CuCl_2$ 50 mg/kg decreased the root length, but in any case the differences were high enough to reach statistical significance. At 100 μM IAA, the interaction with $CuCl_2$ at 100 mg/kg caused a significant reduction of roots, compared with 100 μM IAA+ nCuO at both 50 and 100 mg/kg, and $CuCl_2$ at 50 mg/kg (**Table 3**). The main factor, Cu compounds, shows an increase of 32.5% in root length when treated with nCuO 100 mg/kg (data in appendix **Table S1**).

Table 3 Green pea growth response. From plants that were grown in soil amended in Cu compound (nCuO, bCuO, and $CuCl_2$) at different concentrations (50 and 100 mg/kg of soil) and enhanced with IAA. Results are means ± SE. Letters indicate statistical differences ($p \leq 0.05$).

Treatment			Growth response				
ID	IAA (μM)	Cu Compound (mg/kg of soil)	Stem length (cm)	Root length (cm)	Shoot system length (cm)	Number of leaves	45 day plant survival
1	0	0	17.6 ± 2.0 ab	23.0 ± 2.4 abc	20.5 ± 2.1 a	13 ± 0.9 abcd	4 ± 0.5 ab
2		n50	21.5 ± 2.0 a	26.1 ± 2.4 ab	23.4 ± 2.1 a	14 ± 0.9 abcd	3.5 ± 0.5 ab
3		n100	16.1 ± 2.0 ab	27.8 ± 2.4 ab	18.9 ± 2.1 a	12 ± 0.9 abcd	4.5 ± 0.5 a
4		b50	16.2 ± 2.0 ab	27.7 ± 2.4 ab	18.8 ± 2.1 a	12 ± 0.9 abcd	4.25 ± 0.5 ab
5		b100	16.5 ± 2.0 ab	24.3 ± 2.4 abc	19.2 ± 2.1 a	12 ± 0.9 abcd	4.25 ± 0.5 ab
6		$CuCl_2$ 50	16.1 ± 2.0 ab	19.1 ± 2.4 bc	19.4 ± 2.1 a	11 ± 0.9 abcd	4.25 ± 0.5 ab
7		$CuCl_2$ 100	14.1 ± 2.0 ab	19.4 ± 2.4 bc	17.6 ± 2.1 a	10 ± 0.9 bcd	4.25 ± 0.5 ab
8	10	0	17.0 ± 2.0 ab	19.5 ± 2.4 bc	20.0 ± 2.1 a	11 ± 0.9 abcd	3 ± 0.5 ab
9		n50	19.5 ± 2.0 ab	23.0 ± 2.4 abc	23.2 ± 2.1 a	12 ± 0.9 abcd	3 ± 0.5 ab
10		n100	13.7 ± 2.0 ab	24.3 ± 2.4 abc	16.3 ± 2.1 a	10 ± 0.9 cd	3.25 ± 0.5 ab
11		b50	15.4 ± 2.0 ab	22.1 ± 2.4 bc	18.6 ± 2.1 a	12 ± 0.9 abcd	2.5 ± 0.5 ab
12		b100	17.1 ± 2.0 ab	18.6 ± 2.4 bc	20.2 ± 2.1 a	14 ± 0.9 abcd	4.25 ± 0.5 ab
13		$CuCl_2$ 50	17.4 ± 2.0 ab	23.8 ± 2.4 abc	21.4 ± 2.1 a	13 ± 0.9 abcd	2.75 ± 0.5 ab
14		$CuCl_2$ 100	13.8 ± 2.0 ab	23.9 ± 2.4 abc	17.2 ± 2.1 a	11 ± 0.9 abcd	3.5 ± 0.5 ab
15	100	0	16.9 ± 2.0 ab	23.4 ± 2.4 abc	20.5 ± 2.1 a	13 ± 0.9 abcd	3.25 ± 0.5 ab
16		n50	16.6 ± 2.0 ab	30.5 ± 2.4 ab	19.6 ± 2.1 a	13 ± 0.9 abcd	1.75 ± 0.5 b
17		n100	17.6 ± 2.0 ab	35.1 ± 2.4 a	20.9 ± 2.1 a	15 ± 0.9 abc	2.5 ± 0.5 ab
18		b50	16.4 ± 2.0 ab	23.2 ± 2.4 abc	17.7 ± 2.1 a	15 ± 0.9 ab	3.75 ± 0.5 ab
19		b100	16.5 ± 2.0 ab	24.9 ± 2.4 abc	22.4 ± 2.1 a	16 ± 0.9 a	2.5 ± 0.5 ab
20		$CuCl_2$ 50	10.7 ± 2.0 b	26.1 ± 2.4 ab	14.1 ± 2.1 a	10 ± 0.9 d	2.25 ± 0.5 ab
21		$CuCl_2$ 100	12.9 ± 2.0 ab	12.9 ± 2.4 c	16.1 ± 2.1 a	11 ± 0.9 abcd	3.25 ± 0.5 ab

± represent standard error.

On the other hand, 100 µM IAA+ $CuCl_2$ 50 mg/kg decreased stem length, compared with nCuO 50 mg/kg (no IAA). A previous study showed that 50 meq/l chloride imposed significant toxicity to the whole plant of navy beans, a member of the same family as green pea (Eaton, 1942). Katekar and Geissler (1981) reported that under chloride presence, pea plants form chlorinated indolacetic acids that have higher affinity than IAA for auxin receptor; thus, reducing the IAA activity in plants. In addition, Kolbert et al. (2012) reported that in *Arabidopsis thaliana* (Brassiceae) Cu stress decreased the auxin-dependent gene expression, which resulted in a reduction of root growth.

Copper concentration in green pea plant tissues

In this study, we used agricultural soil amended with different concentrations of Cu compounds and IAA at different levels. Cu concentration in roots, stems, leaves, and pods are shown in **Figure 2**. Cu uptake in roots (**Figure 2a**) had a significant increase (135.7% and 130%) with nCuO at 50 and 100 mg/kg, respectively, in absence of IAA, compared to control. $CuCl_2$ at 100 mg/kg plus 10 µM IAA increased root Cu by 217%, compared to control (**Figure 2a**). In addition nCuO, bCuO, and $CuCl_2$ at 100 mg/kg, plus 100 µM IAA, increased root Cu by 191%, 197%, and 176%, respectively, compared to control. Averages of main factor IAA are shown in **Figure 3a.** This figure shows an increase in root Cu of 58% in treatments with 100 µM IAA. Averages of the main factor, Cu compounds, showed statistical increases in roots Cu, compared with control (**Figure 3e)**.

Figure 2b shows the Cu concentration in stems. Treatment 10 µM IAA+ nCuO 100 mg/kg increased by 83.7%, and treatment 10 µM IAA+ bCuO 50 mg/kg increase by 77.8%, compared to respective control. The average of main factor IAA shows that Cu concentrations in stems

increase by 58.4% in treatments with 100 μM compared to controls (**Figure 3b**). On the other hand, the main factor Cu compounds, showed an increase of 40%, only with nCuO 50 mg/kg, compared to controls.

Cu concentrations in leaves are shown in **Figure 2c.** As seen in this figure, only the combination of 10 μM IAA+ nCuO 100 mg/kg increased Cu in leaves (57%), compared with the respective control. The factorial analysis showed that IAA at the two concentrations, significantly increased leaf Cu, when the average was taken over all Cu treatments (**Figure 3 c).** Increases were of 30% and 24% for 10 and 100 μM, respectively, compared to controls. The main effect for Cu compounds shows that there were no statistical differences (**Figure 3g**). Similarly, there were no statistical differences in pod Cu (**Figure 2d**). Though, the main effect of IAA shows a statistical increase of Cu uptake (67%) with 10 μM IAA, compared to controls (**Figure 3d**). Whereas, the main factor, Cu compounds, did not show any statistical difference (**Figure 3h**).

Concerning the Cu accumulation in tissues, Hong et al. (2015) exposed alfalfa and lettuce to several Cu nanoparticles and compounds and reported that this is affected by the species of plant and the Cu compound. In alfalfa, the highest Cu accumulation was found in roots and stems of plants treated with $CuCl_2$ at 20 mg/L, while in lettuce, the highest Cu concentration was found in roots of plants treated with $CuCl_2$, but in stems there was more Cu in plants treated with nCu. Addition of IAA has shown to increase the uptake of heavy metals by plants. Lopez et al. (2005) reported that Pb uptake in alfalfa plants increase by 20% in plants exposed to 0.2 mM Pb/10 μM IAA; moreover, the root Pb increased to 40% when the IAA concentration increased to 100 μM. Similar results were reported by Fassler et al. (2010) in sunflower (*Helianthus annuus*) exposed to Zn and Pb under different IAA concentrations. Pazurkiewicz-Kocot et al. (2008) mentioned that IAA activates the membrane ATPases, influencing the transport of ions resulting in influx

of some elements. In addition, the Aux/IAA proteins are putative transporters (Nigam and Sawant, 2013) that could be involved in Cu accumulation and transport.

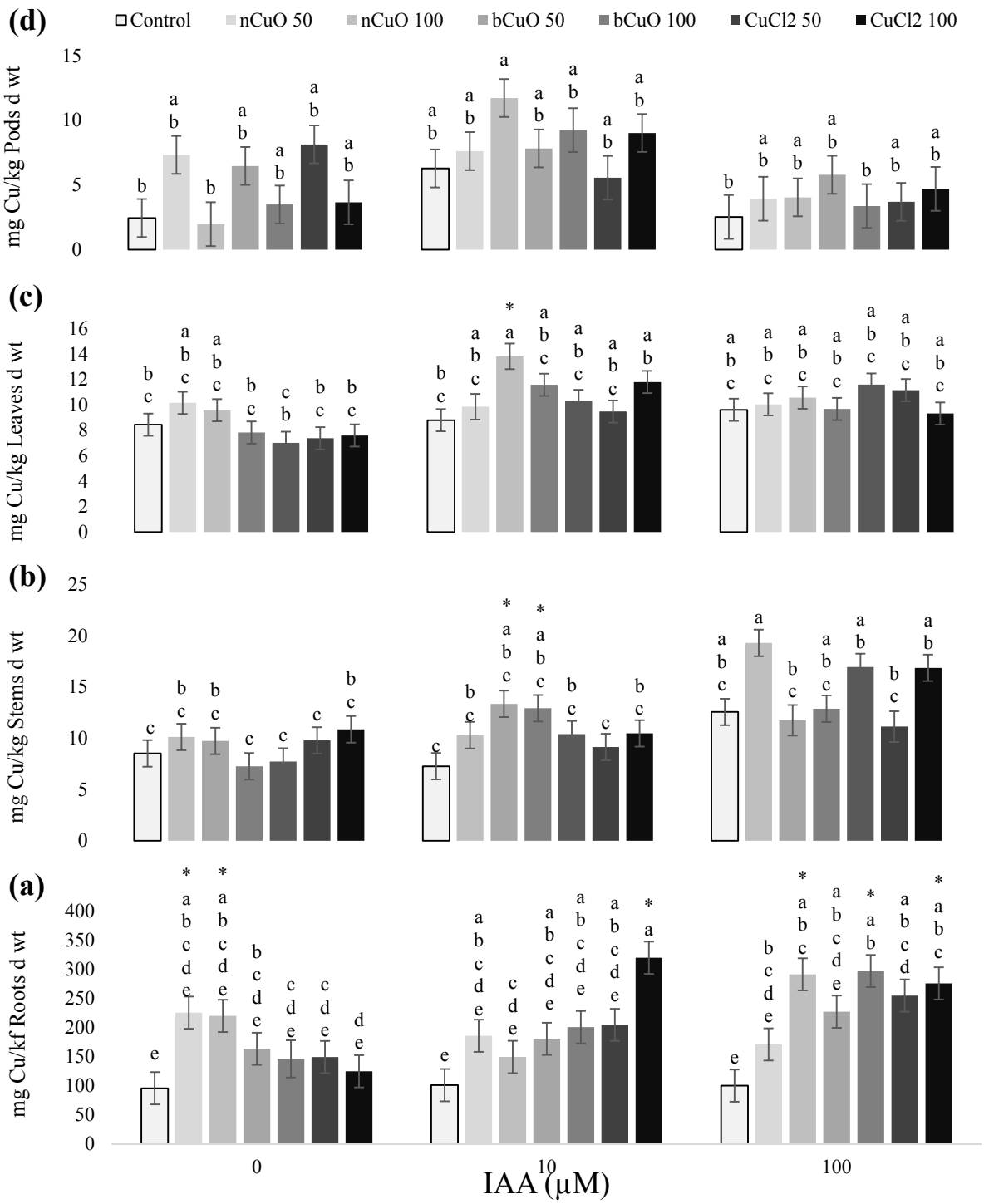

Figure 2. Cu concentration (a) roots, (b) stems, (c) leaves of green pea plants grown to 45 days (beginning of blossom), and (d) pods of green pea plants grown for 90 days (full maturity) in soil amended with nCuO, bCuO, and $CuCl_2$ (corresponding to 50 and 100 mg/kg of Cu) and induced with IAA µM at 0, 10, and 100. Data are expressed in means ± SE, and letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

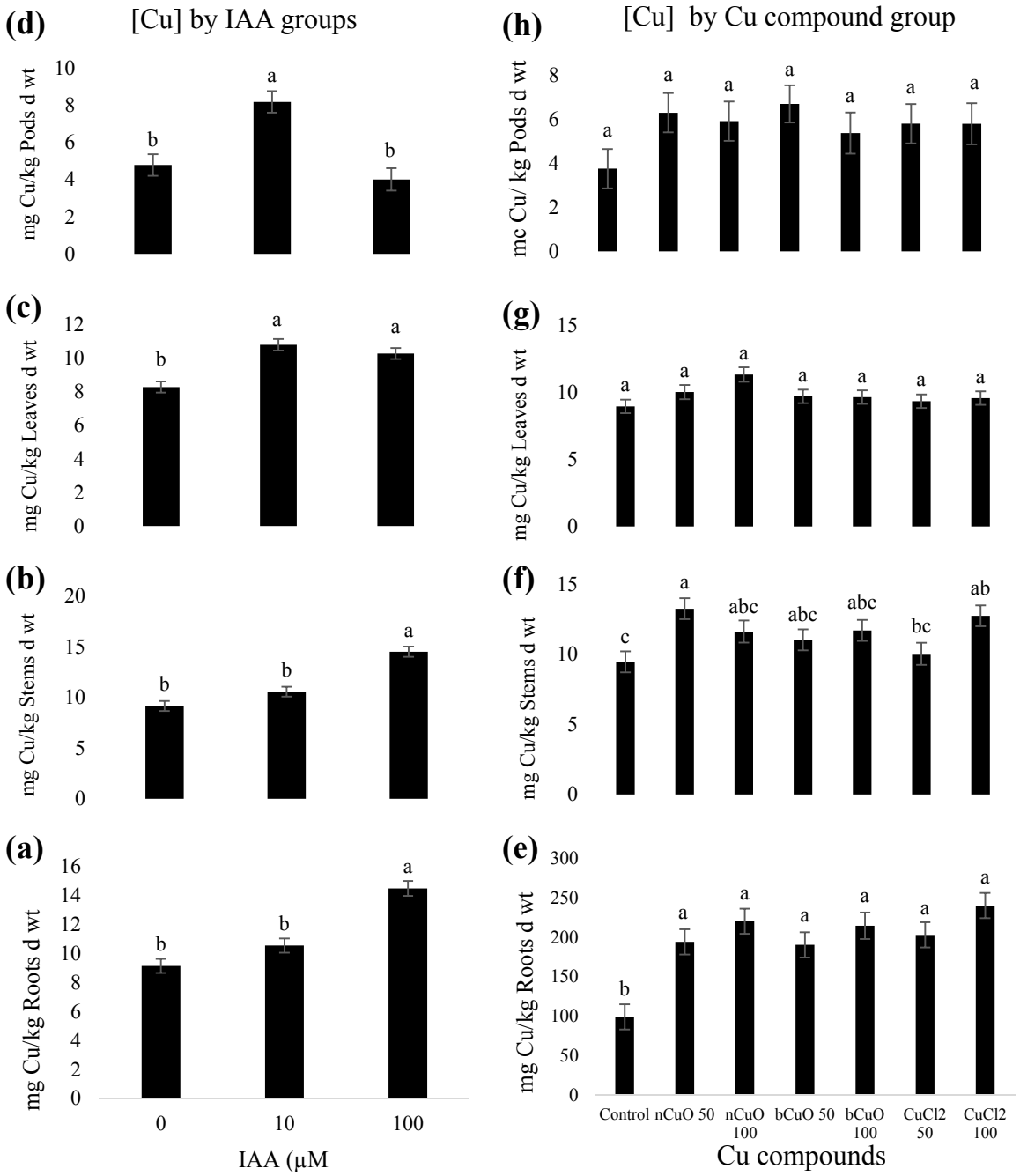

Figure 3. Average Cu concentration as affected by IAA concentrations in (a) roots, and (b) stems, (c) leaves grown at 45 days (beginning of blossom), and (d) pods of green pea plants grown for 90 days (full maturity). Average copper concentration as affected by the copper compound in (e) roots, and (f) stems (g) leaves grown at 45 days, and (h) pods grown at 90 days.

Carotenoid and chlorophyll content

Results for carotenoids, chlorophyll *a*, chlorophyll *b*, and total chlorophyll are shown in **Figure 4**. The two-way ANOVA showed significant differences ($p \leq 0.05$) for the treatment effect, while the Tukey test grouped the averages in three groups (abc). To ease the interpretation, the averages of all treatments were separated by the IAA concentration. Carotenoid content in pea leaves show that in the absence of IAA treatment $CuCl_2$ 100 mg/kg decrease carotenoid content by 48%, compared to control (**Figure 4 a**). In addition, 10 µM of IAA combined with $CuCl_2$ at 50 and 100 mg/kg, resulted in a reduction of 29% and 31%, respectively, compared to the respective control. An analysis of the major factors, Cu compounds, showed that the average of $CuCl_2$ at 50 and 100 mg/kg reduced carotenoids by 23% and 26%, respectively, compared to control (**Figure 5 e)**. On the other hand, **Figure 5 a** shows that at 100 µM IAA, the average of carotenoids, over all Cu treatments, was significantly reduced (11%), compared to control ($p \leq 0.05$). Peng et al. (2013) reported a reduction of carotenoids in *Elsholtzia splendens* (Lamiaceae) treated with $CuSO_4 \cdot 5H_2O$: 0.2 µM.

Treatment effects on chlorophylls were somehow different. Chlorophyll *a* shows statistical reductions of 26% and 36.5% in plants exposed to bCuO at 50 mg/kg and $CuCl_2$ at 100 mg/kg, respectively, compared to control (**Figure 4b)**. $CuCl_2$ at 50 and 100 mg/kg plus 10 µM IAA produced reductions of 37%, and 43%, respectively, compared to control. In addition, 100 µM IAA+ $CuCl_2$ at 50 and 100 mg/kg, reduced chlo-a by 39% and 36%, respectively compared to control (**Figure 4 b)**. The average of main factor IAA at 100 µM, over all Cu concentrations showed a reduction of 17% (**Figure 5b**). On the other hand,

Figure 5f shows that main factor Cu compounds reduced chlo-a by 30% and 38% in plants exposed to $CuCl_2$ at 50 and 100 mg/kg, respectively, compared to controls.

Chlorophyll *b* was quite different from chlorophyll *a,* since all treatments showed reduction, compared to the main control (no IAA, no Cu) (**Figure 4c**). The main effect of factor IAA show that both 10 µM IAA and 100 µM IAA decreased chlo-b by 15% and 29%, respectively, compared to no IAA (**Figure 5c)**. While the effects on chlorophyll *a* followed a similar pattern than carotenoids (**Figure 5 e and f**), the effects on chlorophyll *b* were different (**Figure 5g**). The analysis of major factor (Cu compounds) (**Figure 5g)** showed reductions of 52-55% for $CuCl_2$, compared to control ($p \leq 0.05$). Similar results were found for total chlorophyll (**Figure 4d**), where a reduction was found in all treatments containing Cu, compared to control. Moreover, treatments with $CuCl_2$ at 50 and 100 mg/kg, plus 10 µM IAA, decreased chlo-b by 40% and 44%, compared to their respective control (**Figure 4d**). A comparison between the two concentrations of IA showed that 10 µM IAA reduced chlo-b by 10%, while 100 µM IAA reduced it by 20%, compared to no IAA (**Figure 5d**). Lastly, the main factor Cu compounds showed a decrease in total chlorophyll of 19%, 18%, 37%, and 42% in plants exposed to nCuO 100 mg/kg, bCuO 50 mg/kg, $CuCl_2$ 50 mg/kg, and $CuCl_2$ 100 mg/kg, respectively, compared to controls. Previous studies have shown that excess of Cu reduces the synthesis of protochlorophyllide reductase, which results in a reduction of chlorophyll pigments (Saglam et al., 2016). This was shown in a study with rice (*Oryza sativa*) seedlings, where nCuO reduced carotenoid contents (Kumar Shaw and Hossain, 2013). In support of a study made by Karatas et al. (2010), where it shows that at levels of 10 µ IAA the chlorophyll and carotenoid content decrease in *Tropaeolum majus* L. leaves. In our study, IAA at 10 and 100 µM interacted negatively with $CuCl_2$, reducing total chlorophyll,

chloro-*a,* chloro-*b* and carotenoids. This suggests a lower production of ATP due the ion release of $CuCl_2$. In conclusion, this study agrees with previous studies where the chlorophyll content also decreases with higher levels of Cu compare to the control (Kirbag and Munzuroglu 2005; Aarti et al. 2006; Perreault et al. 2010; Dimkpa et al. 2012)..

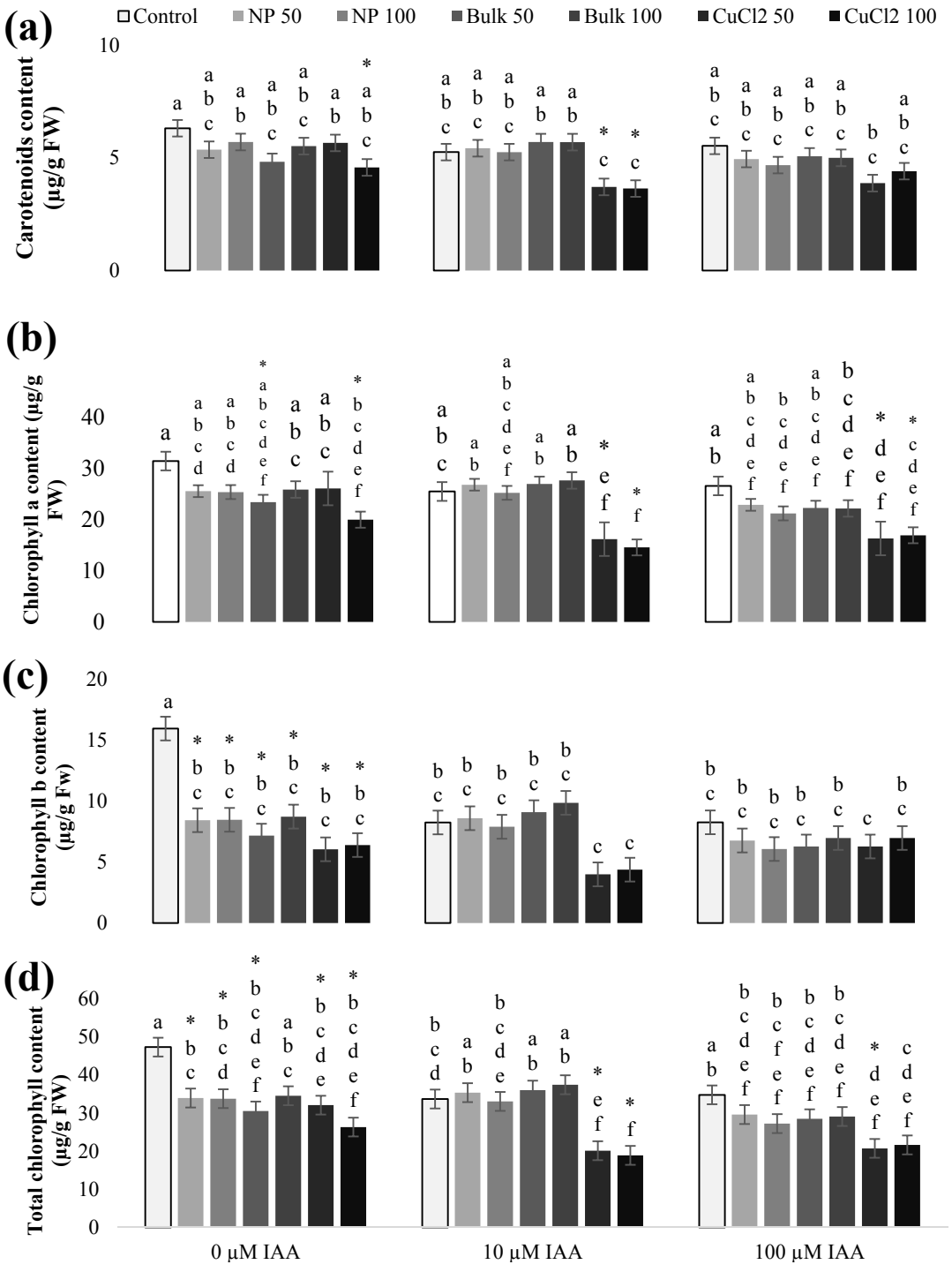

Figure 4. (a) Chlorophyll *a*. **(b)** Chlorophyll *b*. **(c)** Total Chlorophyll content, and **(d)** Carotenoids in leaves of 45 day-old plants grown in soil amended with nCuO, bCuO, and $CuCl_2$ (corresponding to 50 and 100 mg/kg of Cu) and induced with IAA µM at 0, 10, and 100. Data are expressed in means ± SE, and letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

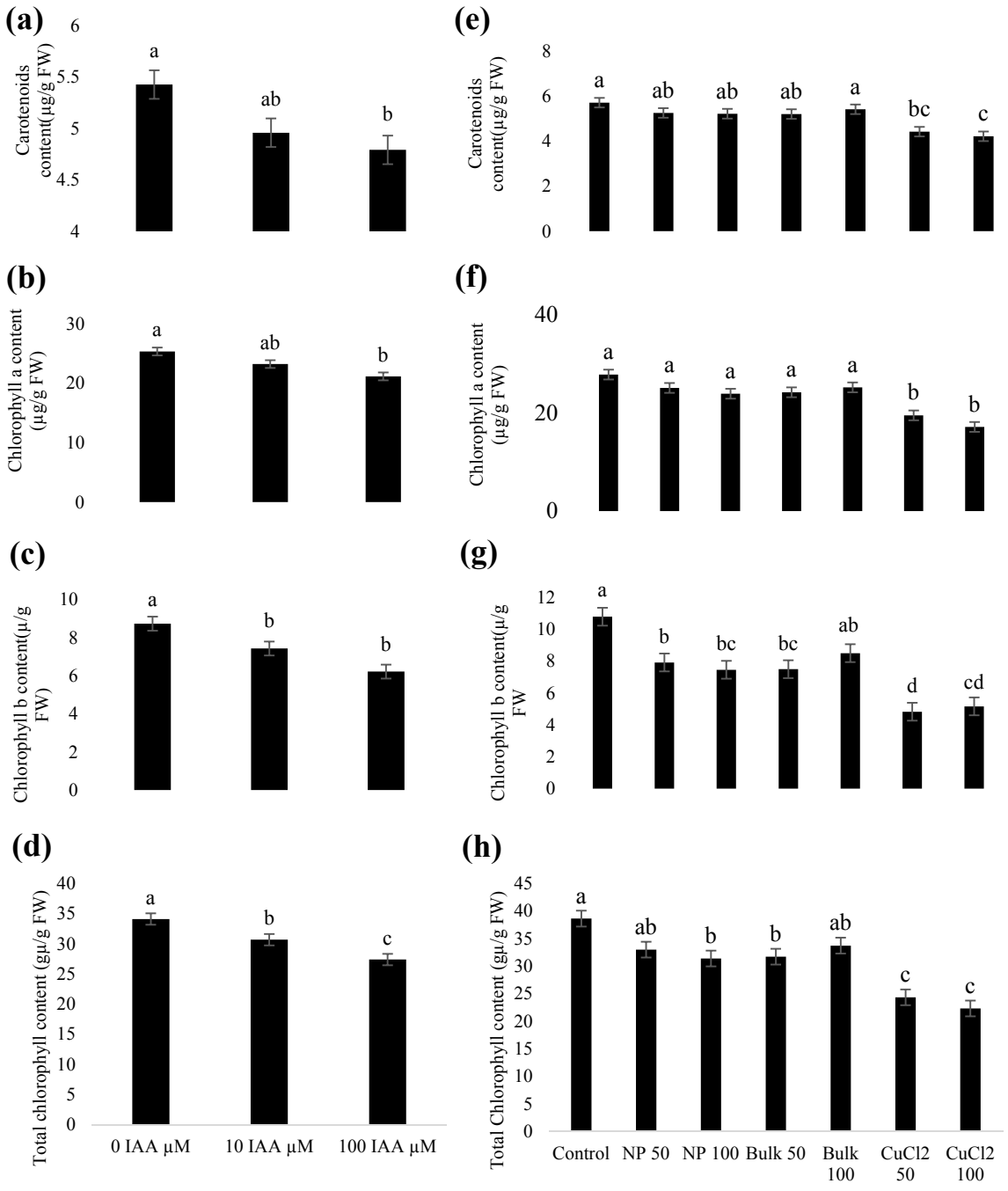

Figure 5. Carotenoids and Chlorophyll in leaves from green pea plants grown in soil amended with nCuO, bCuO, and $CuCl_2$ (corresponding to 50 and 100 mg/kg of Cu) and induced with IAA µM at 0, 10, and 100.for 45 days Averages are grouped by IAA concentrations. (a) Carotenoids (b) Chlorophyll *a*, (c) Chlorophyll *b,* and (d) Total Chlorophyll. Averages are grouped by Cu compound levels in: (e) Carotenoids (f) Chlorophyll *a*, (g) Chlorophyll *b,* and (h) Total Chlorophyll. Data are expressed in means ± SE, and letters indicate statistical differences ($p \leq 0.05$).

Enzymatic activity

The catalase (CAT) activity in green pea plant roots, was recorded in plants grown for 45 days in soil amended with nCuO, bCuO, and $CuCl_2$, and treated with IAA at different concentrations (**Figure 6).** As seen in this figure, 10 µM IAA produced the highest CAT activity (**Figure 6a)**. At 10 µM IAA there was an increase in CAT activity of 74.5%, compared to control (0 µM IAA). Even though CAT activity at 100 µM IAA increased by 59.2% compared with control (no IAA), the difference, was not statistically significant (**Figure 6b**). This suggests that the IAA plays a role in increasing CAT activity by protecting plants against oxidative stress, similar effects were reported by Gangwar et al. (2012), which shows that an addition of 100 µM IAA promotes the regulation of oxidative stress that metals can induce. In the other hand, the analysis of the major factor (Cu compounds) shows that controls have a higher CAT activity when compared to the rest of the treatments (**Figure 6c**). CAT activity was reduced in all treatments with nCuO compared to controls, except in treatment with no IAA and nCuO 50 mg/kg. Similar results were reported by Ye et al. (2014), and Hong et al. (2015), where rice and alfalfa roots showed a reduction in CAT activity due to the Cu at 50 µM and nCuO at50 mg/L, respectively. Agami, R. A., (2016) reported that IAA supports antioxidant systems when exposed to Cu. Conversely, there are studies by Choudhary et al. (2010), Dimkpa et al. (2012), and Nair and Chung (2015) that have reported a higher CAT activity in radish seedlings, wheat, and green pea seedlings that were treated with $CuSO_4$ at 0.2 mM and 50 nm nCuO, and nCuO at 100 and 200 mg/dm³, respectively. Whereas in this study it only showed higher CAT activity with 0 µM IAA + nCuO 50 mg/kg, 10 µM IAA + $CuCl_2$ 50 mg/kg, and 100 µM IAA + nCuO 50 mg/kg.

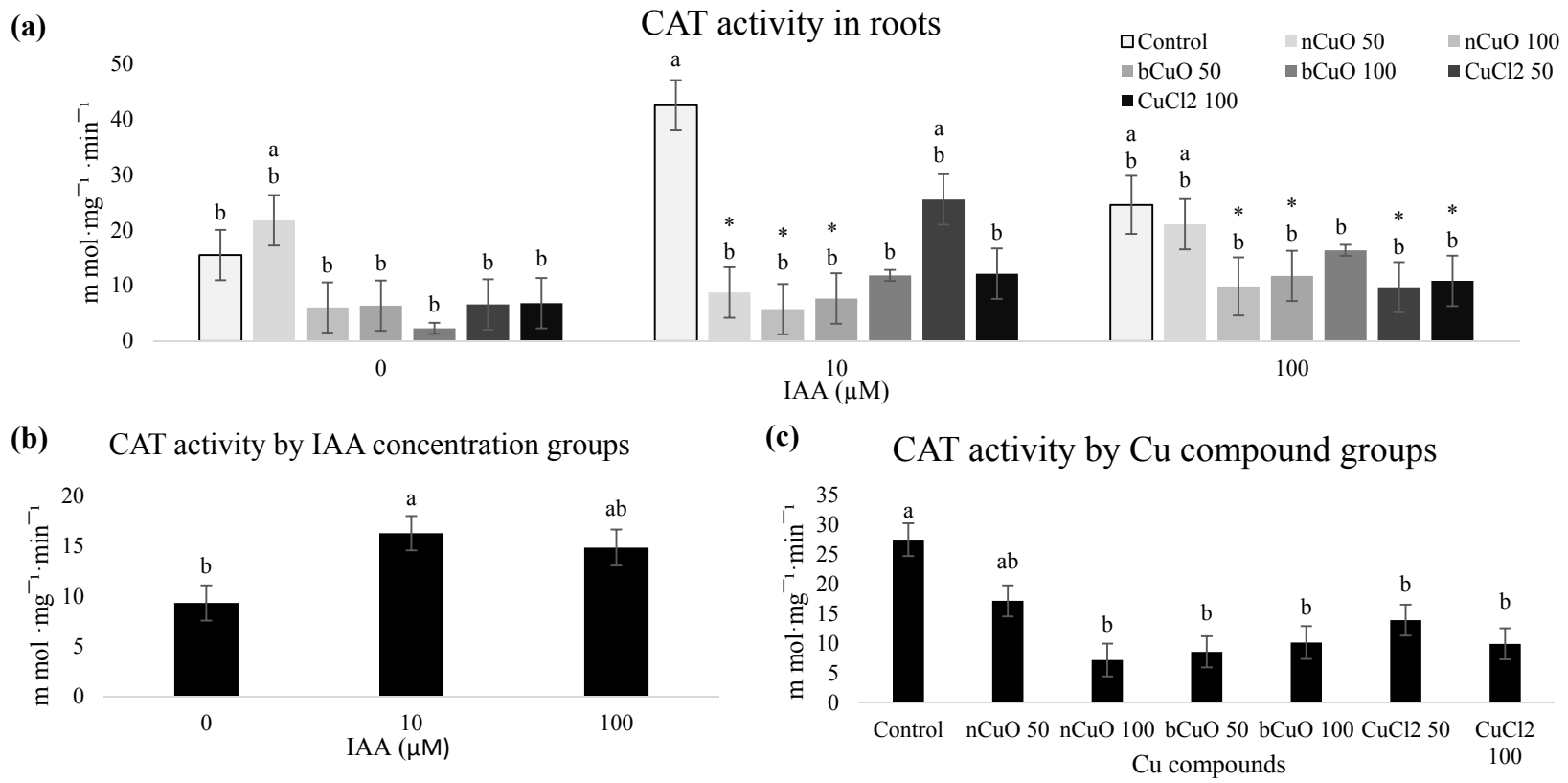

Figure 6 (a) CAT enzymatic activity in green pea roots from plants grown for 45 days in soil amended with a combinations of nCuO, bCuO, and $CuCl_2$ (at 50 and 100 mg/ kg of soil) and IAA at (0,10, and 100 µM). **(b)** Statistical differences in leaf quantity grouped by Cu compound. **(c)** Statistical differences in leaf quantity grouped by IAA (0, 10, and 100) µM content. Results are means ± SE, and statistical differences are indicated with letters and * indicate differences when compared to the respective control ($p \leq 0.05$)

Micro- and Macro-nutrients concentrations in green pea plant tissues

Micro- and macro-nutrients uptake in peas under the conditions of this research did not show any alterations in the uptake of P and K. However, nutrients such as Ca, Mg, S, B, Cu, Fe, Mn, Mo, and Ni were affected. This section contains reports on only essential elements that were affected by the treatments, revealed by the one-way ANOVA analysis (specifically, comparisons against the respective control). Cu increased in treatments with no IAA at nCuO at 50 and 100 mg/kg (136% and 130%), respectively (**Table 4**). This observation suggests that Cu uptake was facilitated by the particle size, consistent with Wang et al. (2012), who reported 1.8 times more Cu in maize roots exposed to nCuO, comparted with bCuO. Treatment with 10 μM IAA and $CuCl_2$ at 100 mg/kg showed an increase in Cu uptake by 217%, compared to control. Treatments with 100 μM IAA and with nCuO, bCuO, and $CuCl_2$, all at 100 mg/kg, increase root Cu by 191%, 197%, and 175%, respectively. This finding suggests that the Cu concentration added to the soil could influence the result. IAA 10 μM plus $CuCl_2$ at 50 and 100 mg/kg increased Ca by 48% and 62%, respectively.

The analysis of stem samples showed that Cu increased by 84% and 77% in plants exposed to 10 μM IAA plus nCuO at 100 mg/kg and bCuO at 50 mg/kg, respectively, compared to control (**Table 5**). Manganese decreased in plants exposed to 10 μM IAA plus nCuO and $CuCl_2$ at 100 mg/kg. Wagenknecht and Burris (1950) reported that Mn is needed as a stimulant for the IAA oxidase activity in pea. It has also been reported that high levels of Cu can induce Mn reduction (Voss 1998). Boron (B) increased by 266%, 222%, and 238% in treatments with 100 μM IAA plus bCuO at 50 and 100 mg/kg, and also plus $CuCl_2$ at 100 mg/kg, respectively, compared to control. This results could suggests that the pH might have

played a role on this, since all treatments had IAA at high concentrations, and even though IAA is a weak acid, it can still induce a change in pH. Lopez et al. (2007) reported that B increased in alfalfa leaves as the IAA concentration increased. In green pea, stem Ca was similar as in roots, since there was an increment in plants exposed to IAA $CuCl_2$ at both 50 and 100 mg/kg, without IAA. Calcium decreased in treatments with 100 µM IAA plus nCuO at 100 mg/kg and bCuO at 100 mg/kg. Dimkpa et al. (2015) reported that 500 mg/kg of nCuO decreased the uptake of Ca and Mn in beans. A decrease of Mg in stem was shown in treatments with 100 µM IAA + nCuO and bCuO, both at 100 mg/kg. Molybdenum (Mo) increased in stems by 85% compared to control, in treatment with 100 µM IAA and bCuO at 100 mg/kg. Le Van et al. (2015) reported that nCuO significantly lowers Mo in transgenic cotton. Magnesium (Mg) in stems was reduced by 35% in treatments with 100 µM IAA + both nCuO and bCuO at 10 mg/kg. Sulfur (S) increased in stems, in treatments without IAA and $CuCl_2$ at both 50 and 100 mg/kg, similar results were reported by Trujillo-Reyes et al. (2014), were S uptake increased in lettuce plants when treated with nCu and nCuO.

In leaves, Cu increased by 57% in plants exposed to 10 µM IAA+ nCuO at 100 mg/kg, compared to respective control (**Table 6**). This result could suggest an effect of particle size, since bCuO did not show effects. Although all Cu treatments, at all IAA levels, showed higher concentrations of Cu than controls, the differences were not significant. Iron was decreased by 100 µM IAA + $CuCl_2$ at 50 and 100 mg/kg (41% and 50 %, respectively), compared to control. Choudhary et al. (2010) reported that Cu reduced total Fe and biomass in radish seedlings. Hong et al. (2015) reported that $CuCl_2$ significantly reduced Fe content in alfalfa and lettuce. In green pea, leaf B increased by 174%, 220%, 234%, and 288% in plant exposed to 100 µM IAA plus nCuO and bCuO, both at 50 and 100 mg/kg, respectively. In

addition, $CuCl_2$ at 100 mg/kg, without IAA, increased leaf B by 885%. Another element that was affected is Ca, which increased throughout all treatments without IAA, except with nCuO. However, it decreased by 36% in plants exposed to 100 μM IAA and $CuCl_2$ at 100 mg/kg. A reduction in Ca has been reported by Trujillo-Reyes et al. (2014) in lettuce plants treated with nC/nCuO. Sulfur in leaves increased by 217% and 264% in treatments without IAA and $CuCl_2$ at both 50 and 100 mg/kg, respectively, compared to control. Similar results were observed in stems. Mg increased by 34%, 36%, 41%, and 52% in leaves of plants exposed to bCuO and $CuCl_2$, both at 50 and 100 mg/kg, in absence of IAA, compared to control.

In pods, nCuO at 50 mg/kg, without IAA, increased Fe by 258% and Ni by 325%, compared to control (**Table 7**). In addition, bCuO at 100 mg/kg also increased Ni by 157%. Moreover, bCuO at 50 and 100 mg/kg, increased B by 47%, compared to the control.

Table 4 Micro- and Macro- nutrients affected in root from plants grown for 45days in soil amended with combinations of IAA (0, 10, and 100 µM) and 50 and 100 mg of Cu from nCuO, bCuO, and $CuCl_2$. Data show averages ± SE of four replicates, and comparisons were made in respect to their respective controls ($p \leq 0.05$).

organ	Affected element	Treatment (mg/kg soil)	Concentration (mg/kg d wt tissue)	%	
Roots	Cu	Control 1 (0 µM IAA)	95.7 ± 27.7	100%	-
		nCuO 50 mg/kg	225.7 ± 27.7	236%	↑
		nCuO 100 mg/kg	220.2 ± 27.7	230%	↑
		Control 2 (10 µM IAA)	101 ± 27.7	100%	-
		10 µM IAA + $CuCl_2$ 100 mg/kg	320.1 ± 27.7	317%	↑
		Control 3 (100 µM IAA)	100.2 ± 27.7	100%	-
		100 µM IAA + nCuO 100 mg/kg	291.6 ± 27.7	291%	↑
		100 µM IAA + bCuO 100 mg/kg	297.4 ± 27.7	297%	↑
		100 µM IAA + $CuCl_2$ 100 mg/kg	276.0 ± 27.7	275%	↑
	Ca	Control 2 (10 µM IAA)	12064 ± 2283	100%	-
		10 µM IAA + $CuCl_2$ 50 mg/kg	17823 ± 2283	148%	↑
		10 µM IAA + $CuCl_2$ 100 mg/kg	19559 ± 2283	162%	↑

Table 5. Micro- and Macro- nutrients affected in stem from plants grown for 45days in soil amended with combinations of IAA (0, 10, and 100 µM) and 50 and 100 mg of Cu from nCuO, bCuO, and $CuCl_2$. Data show averages ± SE of four replicates, and comparisons were made in respect to their respective controls ($p \leq 0.05$).

organ	Affected element	Treatment (mg/kg soil)	Concentration (mg/kg d wt tissue)	%	
Stem	Cu	Control (10 µM IAA)	7.3 ± 1.3	100%	-
		10 µM IAA + nCuO 100 mg/kg	13.4 ± 1.3	184%	↑
		10 µM IAA + bCuO 50 mg/kg	12.9 ± 1.3	177%	↑
	Mn	Control (10 µM IAA)	16.1 ± 0.6	100%	-
		10 µM IAA + nCuO 100 mg/kg	13.1 ± 0.6	81%	↓
		10 µM IAA + $CuCl_2$ 100 mg/kg	12.6 ± 0.6	78%	↓
	B	Control (100 µM IAA)	3.2 ± 1.2	100%	-
		100 µM IAA + bCuO 50 mg/kg	11.7 ± 1.2	366%	↑
		100 µM IAA + bCuO 100 mg/kg	10.3 ± 1.2	322%	↑
		100 µM IAA + $CuCl_2$ 100 mg/kg	10.8 ± 1.2	338%	↑

	Mo	Control (100 µM IAA)	44.6 ± 6.0	100%	-
		100 µM IAA + bCuO 100 mg/kg	82.5 ± 6.0	185%	↑
	Ca	Control (0 µM IAA)	14671 ± 1082	100%	-
		$CuCl_2$ 50 mg/kg	19478 ± 1082	133%	↑
		$CuCl_2$ 100 mg/kg	23920 ± 1250	163%	↑
		Control (100 µM IAA)	18121 ± 1082	100%	-
		100 µM IAA + nCuO 100 mg/kg	12328 ± 1082	68%	↓
		100 µM IAA + bCuO 100 mg/kg	11389 ± 1082	63%	↓
	Mg	Control (100 µM IAA)	5823 ± 371	100%	-
		100 µM IAA + nCuO 100 mg/kg	3757 ± 371	65%	↓
		100 µM IAA + bCuO 100 mg/kg	3767 ± 371	65%	↓
	S	Control (0 µM IAA)	3676 ± 596.2	100%	-
		$CuCl_2$ 50 mg/kg	7059 ± 596.2	193%	↑
		$CuCl_2$ 100 mg/kg	7998 ± 596.2	218%	↑

Table 6 Micro- and Macro- nutrients affected in leaves from plants grown for 45days in soil amended with combinations of IAA (0, 10, and 100 µM) and 50 and 100 mg of Cu from nCuO, bCuO, and $CuCl_2$. Data show averages ± SE of four replicates, and comparisons were made in respect to their respective controls ($p \leq 0.05$).

organ	Affected element	Treatment (mg/kg soil)	Concentration (mg/kg d wt tissue)	%	
Leaves	Cu	Control (10 µM IAA)	8.8 ± 0.9	100%	-
		10 µM IAA + nCuO 100 mg/kg	13.8 ± 1.0	157%	↑
	Fe	Control (100 µM IAA)	117.5 ± 10.6	100%	-
		100 µM IAA + $CuCl_2$ 50 mg/kg	69.6 ± 10.6	59%	↓
		100 µM IAA + $CuCl_2$ 100 mg/kg	58.7 ± 10.6	50%	↓
	B	Control (0 µM IAA)	2.0 ± 6.9	100%	-
		$CuCl_2$ 100 mg/kg	19.7 ± 6.0	985%	↑
		Control (100 µM IAA)	28.8 ± 6.0	100%	-
		100 µM IAA + nCuO 50 mg/kg	78.8 ± 6.0	274%	↑
		100 µM IAA + nCuO 100 mg/kg	92.2 ± 6.0	320%	↑
		100 µM IAA + bCuO 50 mg/kg	96.2 ± 6.0	334%	↑
		100 µM IAA + bCuO 100 mg/kg	111.7 ± 6.0	388%	↑
	Ca	Control (0 µM IAA)	35226 ± 3235	100%	-
		bCuO 50 mg/kg	50860 ± 3235	145%	↑
		bCuO 100 mg/kg	51215 ± 3235	145%	↑
		$CuCl_2$ 50 mg/kg	52008 ± 3235	148%	↑
		$CuCl_2$ 100 mg/kg	60660 ± 3235	172%	↑
		Control (100 µM IAA)	51861 ± 3235	100%	-

	100 µM IAA + $CuCl_2$ 100 mg/kg	33013 ± 3235	64%	↓
Mg	Control (0 µM IAA)	4506 ± 380	100%	-
	bCuO 50 mg/kg	6041 ± 329	134%	↑
	bCuO 100 mg/kg	6125 ± 329	136%	↑
	$CuCl_2$ 50 mg/kg	6336 ± 329	141%	↑
	$CuCl_2$ 100 mg/kg	6830 ± 329	152%	↑
	Control (100 µM IAA)	5684 ± 329	100%	-
	100 µM IAA + nCuO 50 mg/kg	7095 ± 329	125%	↑
S	Control (0 µM IAA)	2623 ± 747.9	100%	-
	$CuCl_2$ 50 mg/kg	8328 ± 747.9	317%	↑
	$CuCl_2$ $CuCl_2$ 100 mg/kg	9554 ± 747.9	364%	↑

Table 7 Micro- and Macro- nutrients affected in pods from plants grown for 90 days in soil amended with combinations of IAA (0, 10, and 100 µM) and 50 and 100 mg of Cu from nCuO, bCuO, and $CuCl_2$. Data show averages ± SE of four replicates, and comparisons were made in respect to their respective controls ($p \leq 0.05$).

organ	Affected element	Treatment (mg/kg soil)	Concentration (mg/kg d wt tissue)	%	
Pods	Fe	Control (0 µM IAA)	11.9 ± 5.1	100%	-
		nCuO 50 mg/kg	42.6 ± 5.1	358%	↑
	B	Control (0 µM IAA)	25.9 ± 2.9	100%	-
		$CuCl_2$ 50 mg/kg	38.2 ± 2.9	147%	↑
		$CuCl_2$ 100 mg/kg	38 ± 2.9	147%	↑
	Ni	Control (0 µM IAA)	0.4 ± 0.2	100%	-
		nCuO 50 mg/kg	1.7 ± 0.2	425%	↑
		bCuO 100 mg/kg	1.5 ± 0.2	375%	↑

Dry biomass production

The dry biomass of roots was not calculated because in some treatments, even flooded/washed for about 15 min, it was not possible to separate and recover all the roots from some soil fragments. Thus, there should be biased comparisons. Stem dry biomass was only affected by 100 µM IAA + bCuO 100 mg/kg, which showed an increase of 95%, compared to control **(Table 8)**. Factorial analyses showed no effects of main factor IAA

(**Table 9**); however, the other main factor, Cu compounds, showed an effect (**Table 10**). The analysis of interactions showed that bCuO 100 mg/kg, at all levels of IAA, increased stem dry biomass, compared to their controls.

Even though there were no statistical differences in leaf dry biomass (**Table 8**), the main factor analysis (IAA) showed a reduction of 17% in dry biomass at10 µM IAA (**Table 9**). IAA at 10 µM resulted in a reduction (34%) in pod biomass (**Table 9)**. There are controversies in the response of plants to nCuO. Lopez et al. (2007c) reported that the increase of IAA concentrations induce the biosynthesis of ethylene, this reduces the root growth. Nair et al. (2015) reported that nCuO in the range of 100-500 mg/L, reduced green pea plant growth. Dimkpa et al. (2012) reported that there was an increase in biomass on wheat when exposed with nCuO at 500 mg/kg for 14 days. Dimkpa et al. (2012) also reported that both the nCuO and bCuO release similar amount of Cu ions (about 3 mg/L). Thus, the response observed in green pea was not associated with the presence of Cu ions or the level of Cu exposure. Dimkpa et al. (2012) also reported that bCuO induced proliferation of lateral roots in wheat; it is possible that bCuO promoted the production of lateral roots in pea plants, which resulted in an increase in dry biomass. Gangwar et al. (2011), reported that 100 µM IAA decrease pea seedlings growth. However, there are no studies involving exogenous IAA and nCuO; thus, these results cannot be compared with previous results.

Table 8 Dry biomass average (g) from green pea organs grown in: 1) control, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)$CuCl_2$ 50 mg/kg, 7)$CuCl_2$ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + $CuCl_2$ 50 mg/kg, 14) 10 µM IAA + $CuCl_2$ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + $CuCl_2$ 50 mg/kg, and 21) 100 µM IAA + $CuCl_2$ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

			Dry biomass tissue (g)		
ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems
1	0	0	0.72 ± 0.07 abc	1.01 ± 0.11 a	0.25 ± 0.03 abc
2		n50	0.52 ± 0.07 abcde	0.94 ± 0.11 a	0.29 ± 0.03 ab
3		n100	0.60 ± 0.07 abcde	0.93 ± 0.11 a	0.27 ± 0.03 ab
4		b50	0.52 ± 0.07 abcde	0.71 ± 0.11 a	0.22 ± 0.03 bc
5		b100	0.62 ± 0.07 abcde	0.94 ± 0.11 a	0.27 ± 0.03 ab
6		$CuCl_2$ 50	0.64 ± 0.07 abcde	0.86 ± 0.11 a	0.24 ± 0.03 abc
7		$CuCl_2$ 100	0.74 ± 0.07 ab	0.72 ± 0.11 a	0.21 ± 0.03 bc
8	10	0	0.37 ± 0.07 cde	0.58 ± 0.11 a	0.15 ± 0.03 bc
9		n50	0.35 ± 0.07 de	0.67 ± 0.11 a	0.17 ± 0.03 bc
10		n100	0.56 ± 0.07 abcde	0.56 ± 0.11 a	0.18 ± 0.03 bc
11		b50	0.38 ± 0.07 cde	0.52 ± 0.11 a	0.15 ± 0.03 bc
12		b100	0.44 ± 0.08 bcde	0.81 ± 0.11 a	0.23 ± 0.03 bc
13		$CuCl_2$ 50	0.44 ± 0.07 abcde	0.93 ± 0.11 a	0.29 ± 0.03 ab
14		$CuCl_2$ 100	0.33 ± 0.07 e	0.96 ± 0.11 a	0.30 ± 0.03 ab
15	100	0	0.59 ± 0.07 abcde	0.56 ± 0.11 a	0.21 ± 0.03 bc
16		n50	0.60 ± 0.08 abcde	0.48 ± 0.11 a	0.09 ± 0.03 c
17		n100	0.66 ± 0.07 abcde	0.85 ± 0.11 a	0.27 ± 0.03 ab
18		b50	0.80 ± 0.07 a	0.90 ± 0.11 a	0.30 ± 0.03 ab
19		b100	0.73 ± 0.07 abc	1.02 ± 0.11 a	0.41 ± 0.03 a*
20		$CuCl_2$ 50	0.73 ± 0.07 abc	0.50 ± 0.11 a	0.18 ± 0.03 bc
21		$CuCl_2$ 100	0.70 ± 0.08 abcd	0.92 ± 0.11 a	0.25 ± 0.03 abc

Table 9 Dry biomass grouped by IAA (µM) concentrations in green pea organs grown in soil amended with different Cu compounds (nCuO, bCuO, and $CuCl_2$) at different levels and enhanced with IAA. Results are means ± SE. Letters indicate statistical differences ($p \leq 0.05$).

Dry biomass grouped by IAA (μM) concentrations			
IAA concentration	Pod averages	Leaves averages	Stem averages
0 μM IAA	0.62 ± 0.025 a	0.87 ± 0.04 a	0.25 ± 0.012 a
10 μM IAA	0.41 ± 0.026 b	0.72 ± 0.04 b	0.21 ± 0.012 a
100 μM IAA	0.69 ± 0.026 a	0.75 ± 0.04 ab	0.24 ± 0.012 a

Table 10 Dry biomass of green pea organs grouped by Cu compound levels (nCuO, bCuO, and $CuCl_2$) at different levels and enhanced with IAA. Results are means ± SE. Letters indicate statistical differences ($p \leq 0.05$).

Cu Compound levels	Dy biomass grouped by Cu compounds		
	Pod averages	Leave averages	Stem averages
Control (0)	0.56 ± 0.038 a	0.71 ± 0.062 a	0.20 ± 0.019 b
nCuO 50	0.49 ± 0.040 a	0.70 ± 0.062 a	0.18 ± 0.019 b
nCuO 100	0.61 ± 0.038 a	0.78 ± 0.062 a	0.24 ± 0.019 ab
bCuO 50	0.56 ± 0.038 a	0.71 ± 0.062 a	0.22 ± 0.019 ab
bCuO 100	0.59 ± 0.040 a	0.92 ± 0.062 a	0.30 ± 0.019 a
$CuCl_2$ 50	0.60 ± 0.038 a	0.76 ± 0.062 a	0.24 ± 0.019 ab
$CuCl_2$ 100	0.59 ± 0.040 a	0.86 ± 0.062 a	0.25 ± 0.019 ab

Summary

Overall, Cu concentrations in pea tissues were greater in plants exposed to 100 μM IAA, except in pods, which increased in plants exposed to 10 μM IAA. A decrease in pod (34% less) and leaf (17% less) biomass was observed in treatments with 10 μM IAA. Main factor analyses showed that nCuO at 100 mg/kg increased root length by 32.5%, compared with controls. The analysis of the main factor, Cu compounds, showed that $CuCl_2$ at the two concentrations, averaged over the IAA doses, reduced total chlorophyll, chlo-*a*, chlo-*b*, and carotenoids. On the other hand, the interaction of IAA at 10 μM × nCuO at 50 mg/kg produced a numerical increase in total chlorophyll, chlo-*a*, chlo-*b*, and carotenoids; however,

the differences were not statistically significant. In addition, main factor IAA at 10 μM increased CAT activity by 74.5% in roots.

All treatments affected the concentration of micro- and macroelements in tissues. In general the highest Cu treatment concentration (100 mg/kg) increased Cu in roots. $CuCl_2$ at both concentrations, plus IAA at 10 μM, increased Ca in roots. In stem, concentrations of Cu, B, Mo, and S increased with several combination treatments. However, Mn, Mg and Ca, decreased, mainly in plants exposed to bCuO and nCuO. In leaves, there was an increase in Cu, B, Ca, Mg, and S, while there was a decrease in Fe. Finally, nCuO at 50 mg/kg increased pods Fe and Ni; bCuO 100 mg/kg also increased pod Ni, while $CuCl_2$ at both concentrations increased pod B.

These results suggest that the combination of Cu NPs or compounds with IAA alter plan ionome. More studies are needed in order to determine benefits/threats of an increase of essential elements in green pea pods.

Chapter 3: Nutritional values in green pea (*Pisum sativum* L.) seeds from plants exposed to CuO nanoparticles and indole-3acetic acid

INTRODUCTION

Little is known about the effects of NPs in food quality. Studies have reported that NPs such as ZnO inhibit seed production and reduce Fe and biomass in soybean and zucchini, inhibit catalase (CAT) and ascorbate peroxidase (APOX) activity in green pea plants and increase the sucrose concentration in green pea seeds (Yoon et al. 2014; Peralta-Videa et al. 2014; Stampoulis et al. 2009; Mukherjee et al. 2013; Mukherjee et al. 2016). Additionally, nTiO2 has also been reported to reduce wheat biomass (Du et al. 2011). Nevertheless, nCeO2 has been reported to change rice fatty acids and starch, and down-regulated phaseolin and lectins in beans, and reduces CAT activity in tomatoes (Rico et al. 2013, Majumdar et al. 2015; Barrios et al. 2015). Other reports have shown that nCuO reduces growth and increases ROS in green pea, decreases weight in soybean, mug bean, and rice, while it also decreases the shoot length in cilantro (Nair and Chung 2014; Nair and Chung 2015; Hong et al. 2015; Zuverza-Mena et al. 2015).

Nutritional values of legumes have been widely evaluated in many different studies (Durati and Gius 1997; Shewry et al. 1995; Iqbal et al. 2006; Khattab et al 2009). To the author's knowledge, there are no reports on the effects of nCuO in green pea seed nutritional quality, under IAA exposure. The objectives of this study were to determine the effects of nCuO, bCuO, and $CuCl_2$, plus IAA at 0, 10, and 100 μM on green pea seeds. Number of seeds, biomass, crude protein, bioaccumulation of Cu, and other essential elements in seeds were determined.

MATERIALS AND METHODS

Green pea plants were grown for 90 days (full maturity), and seeds were harvested likewise the rest of the plant tissues as mentioned in chapter 2.

Macro- and Micro-nutrients, and Al determination in dry green pea seeds

The digestion of seeds was performed as the rest of the tissues. Briefly, 0.2 g of dried seed samples were digested with 4 mL of NHO_3 (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, S and, Al by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer optima 4300 DV). For quality assurance/quality control, blanks, spikes, and certified standard reference material for plant (spinach leaves NIST-SRM 1570a) were analyzed, obtaining 98% recovery for Cu, with a detection limit of 1.8 µg/L. In addition, 0.5 mg/L multi-elemental standard was analyzed every 10 samples to monitor the matrix effect on the analytes and for quality assurance/quality control (Majumdar et al., 2014).

Crude protein analysis in seeds

Seeds were analyzed for crude protein, regarding total nitrogen content. Seed samples were oven dried for three days at 60^{o} C. Dried seeds were then powdered with mortar and pestle, and sieved in a 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). A sample of 0.1 g of seeds, a K_2SO_4 –catalyst mixture (~1.5 g) and 6 mL of concentrated sulfuric acid (H_2SO_4) were added in a Kjeldahl digestion flask and heated at 175^{o} C for 1 h. Then, the samples were heated again at 375^{o} C for 2 h to ensure a complete digestion, cooled, added 20

mL of distilled water, and hand shaken. The distilled sample was collected, added 40 mL of 10 N sodium hydroxide (NaOH), and placed in the distillation unit. Subsequently, the sample was added 10 mL boric acid (BH_3O_3) containing an indicator; then, 50 mL of distilled were collected and titrated with 0.05 N H_2SO_4 (Bremner, 1996), until the blue colour changed to rose colour. The crude protein content was calculated as % N x 6.25 (Allen, 2002).

Statistical analysis

All the analyses were performed in four replicates per treatment allocated in a completely randomized design. A one-way ANOVA was carried out to analyze treatments against control. Whereas, 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). These were followed by a Tukey's multiple comparisons test with a probability of $p \leq 0.05$.

RESULTS AND DISCUSSION

Seed production

Green pea seeds production is shown in **Table 11.** As seen in this table, there were no effects on seed dry weight; however, the number of seeds per treatment was reduced by some of the treatments. The highest average was found in control plants (no Cu, no IAA). In addition, in absence of IAA, only bCuO at 50 mg/kg significantly reduced the average number of seeds (~52%), compared with control. The addition of 10 µM IAA caused a general reduction in seed dry weight. The two-way ANOVA (Table 11) showed that the treatments: 10 µM IAA alone, and 10 µM IAA plus nCuO at 50 mg/kg, plus bCuO at 100 mg/kg, and plus $CuCl_2$ at 100 mg/kg, significantly reduced the average number of seeds.

Unfortunately, no reference to the effect of IAA in grain formation and seed weight was found in the literature to compare with these results. Simmonds (1987) reported that in *Streptocarpus nobilis* (C. B. Clarke) a short-day plant, manipulated to induce in vitro flowering, IAA inhibited flowering in non-inductive photo-periods. 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 in maize (*Zea mays*), there is tryptophan-dependent synthesis of IAA in developing seeds. There is the possibility that the Cu treatments increase 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.

Table 11 Effects on Seeds. From plants that were grown in soil amended in Cu compound (nCuO, bCuO, and $CuCl_2$) at different concentrations (50 and 100 mg/kg of soil) and enhanced with IAA. Average of seed, and dry weight per treatment have results are means ± SE. Letters indicate statistical differences ($p \leq 0.05$).

	Treatments		Effect on seeds			
ID	IAA (μM)	Cu Compound (mg/kg d wt)	Total seeds per treatment	Average seeds per treatment	Average seed dry weight per treatment (g)	Dry weight of 10 seeds (g)
1	0	0	58	14.5 ± 1.3 a	2.1 ± 0.2 abc	1.43
2		n50	36	9.0 ± 1.3 abc*	1.4 ± 0.2 abc	1.58
3		n100	47	11.8 ± 1.3 abc	1.8 ± 0.2 abc	1.54
4		b50	28	7.0 ± 1.3 bc*	1.4 ± 0.2 abc	2.05
5		b100	45	11.3 ± 1.3 abc	2.1 ± 0.2 abc	1.89
6		$CuCl_2$ 50	42	10.5 ± 1.3 abc	1.8 ± 0.2 abc	1.72
7		$CuCl_2$ 100	47	11.8 ± 1.3 abc	1.9 ± 0.2 abc	1.63
8	10	0	25	6.3 ± 1.3 bc	1.2 ± 0.2 abc	2
9		n50	25	6.3 ± 1.3 bc	1.2 ± 0.2 abc	2
10		n100	31	7.8 ± 1.3 abc	1.3 ± 0.2 abc	1.67
11		b50	35	8.8 ± 1.3abc	1.6 ± 0.2 abc	1.84
12		b100	20	5.0 ± 1.3 c	1.1 ± 0.3 bc	1.66
13		CuCl2 50	31	7.8 ± 1.3 abc	1.5 ± 0.2 abc	1.92
14		CuCl2 100	25	6.3 ± 1.3 bc	1.1 ± 0.2 c	1.71
15	100	0	45	11.3 ± 1.3 abc	2.2 ± 0.2 abc	1.99
16		n50	33	11.0 ± 1.5 abc	2.1 ± 0.3 abc	1.94
17		n100	51	12.8 ± 1.3 ab	2.3 ± 0.2 a	1.79
18		b50	50	12.5 ± 1.3 ab	2.0 ± 0.2 abc	1.57
19		b100	50	12.5 ± 1.3 ab	2.2 ± 0.2 abc	1.73
20		CuCl2 50	47	11.8 ± 1.3 abc	2.0 ± 0.2 abc	1.69
21		CuCl2 100	36	12.0 ± 1.5 ab	2.3 ± 0.3 ab	1.88

± represent standard error.

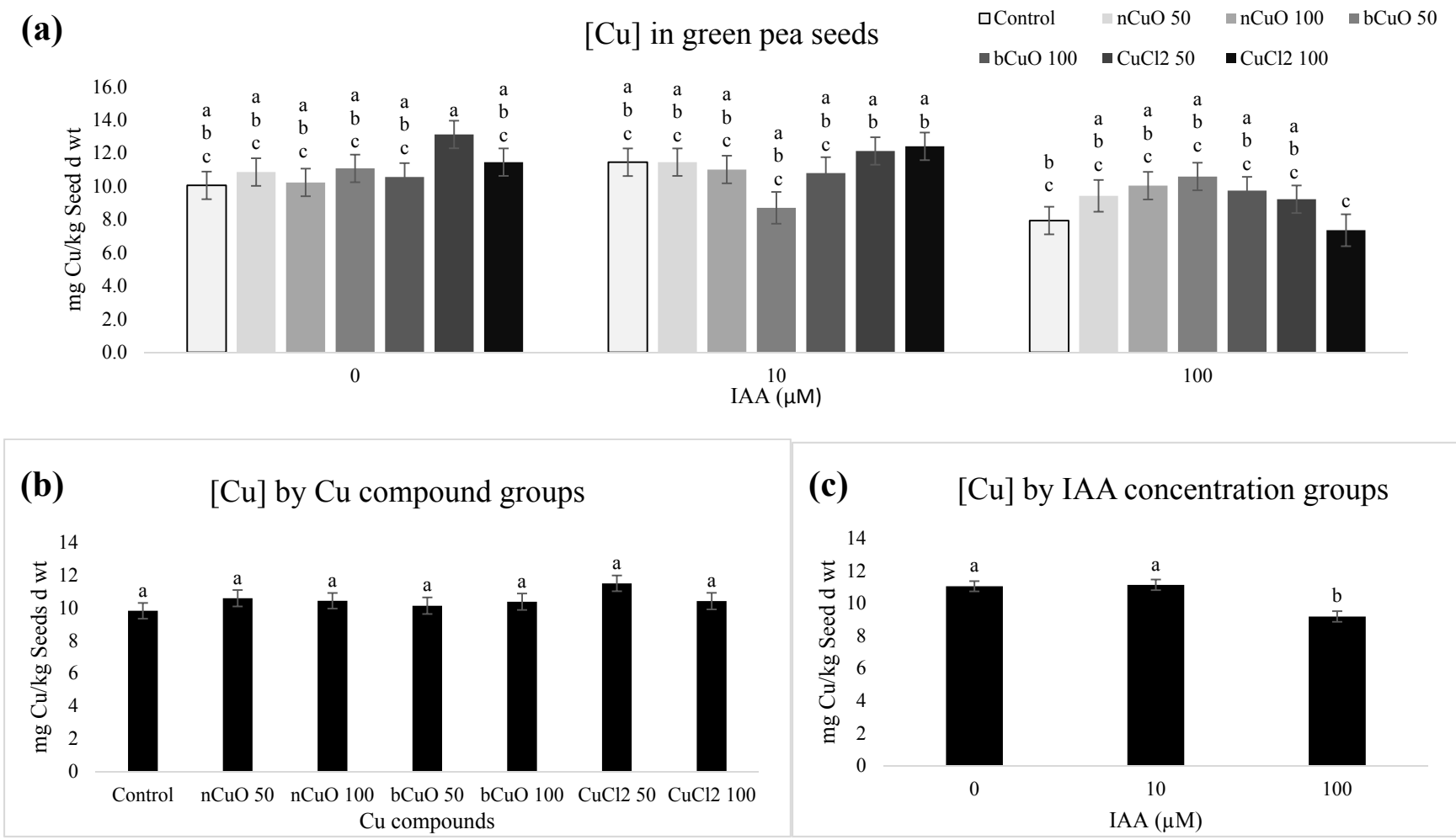

Figure 7 (a) [Cu] in green pea seeds by treatment, from plants that were grown for 90 days in soil amended with at different concentrations of nCuO, bCuO, and CuCl2 and enhanced with IAA (0, 10, and 100 µM). **(b)** [Cu] in green pea seeds when grouped by Cu compounds. **(c)** [Cu] in green pea seeds when grouped by IAA (µM) concentration levels. Results are means ± SE, and statistical differences are indicated with letters and * indicate differences when compared to the respective control ($p \leq 0.05$)..

Copper concentration in seeds

Concentrations of Cu in seeds are shown in Figure 7. The two-way ANOVA showed no effects of the interaction Cu × IAA (Figure 7a). Similarly, the main factor analysis showed no effects of the Cu treatments (Figure 7b); 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 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 Lopez-Moreno et al. (2007), who found an increased in Pb uptake and translocation in alfalfa exposed to 100 μM IAA and Pb at 40 mg/L. This could be an effect of plant species or the exposure method. Lopez-Moreno et al. (2007) performed their experiments in hydroponics and the current study was performed in soil. Mores studies are needed to fully understand the interaction of Cu with IAA. Cu is known to stay at the majority in the root level, since it is the most immobile micro-nutrient in plants (Rusjan 2012). Dimkpa et al. (2012) reports that IAA is an important microbe-plant signaling relation this acts as a beneficial effect to enhance the plant resistance to stress. This suggests that IAA is alleviating the stress that Cu compounds might have cause. These results also suggest that the translocation of Cu in pea is similar independently from the Cu size, and compound added.

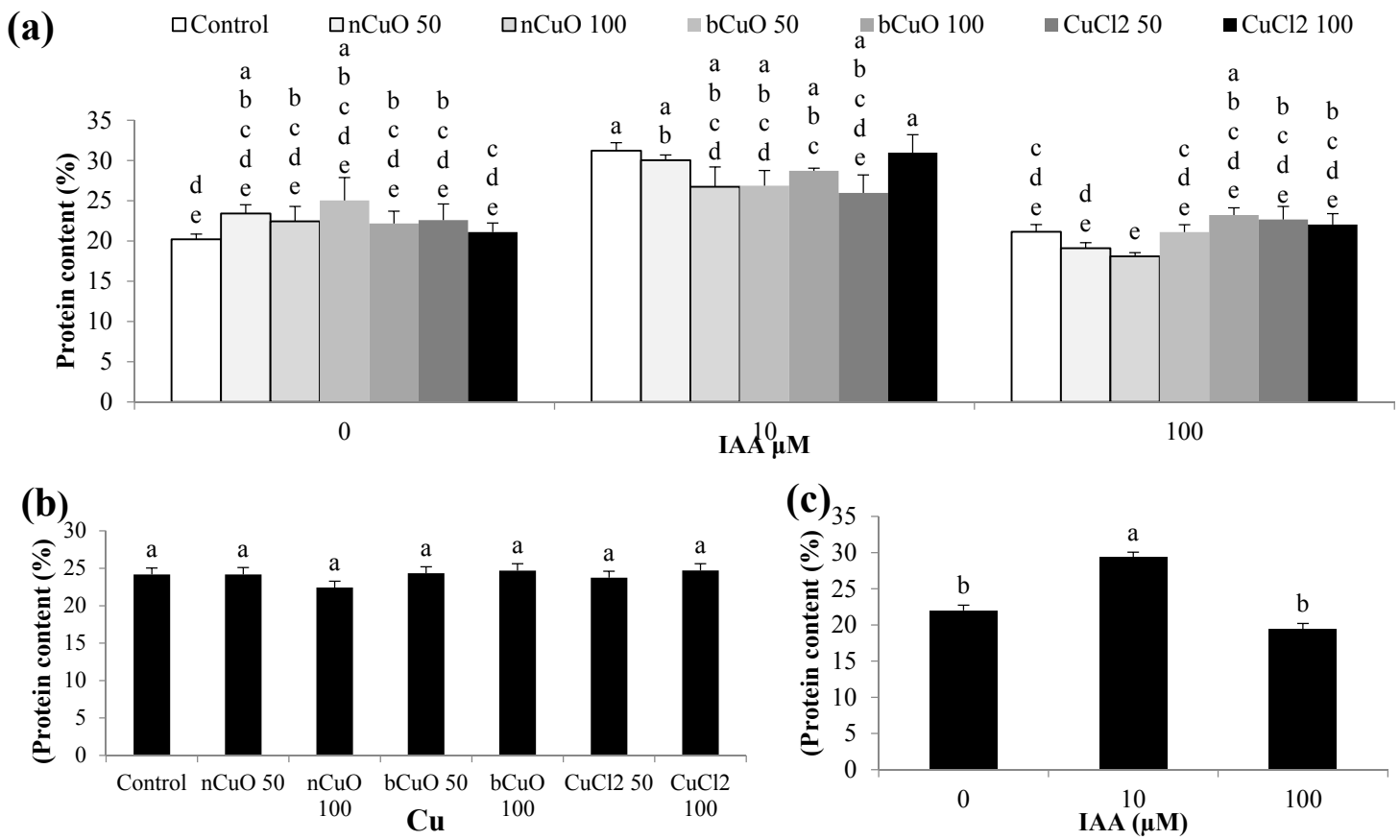

Figure 8 (a) Green pea protein content (%) by treatment, grown for 90 days in soil amended with a total of 21 different combinations of Cu and IAA. **(b)** Statistical differences in leaf quantity grouping the treatments by Cu content. **(c)** Statistical differences in leaf quantity grouping the treatments by IAA (0, 10, and 100) µM content. Results are means ± SE, and statistical differences are indicated with letters and * indicate differences when compared to the respective control ($p \leq 0.05$)

Protein content in seeds

The data for protein content in seeds is shown in Figure 8. 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 (figure 8a). The main factor analysis showed no effect of the Cu treatments (Figure 8b); however, the IAA treatments showed an increased in seed protein at 10 µM. Gangwar et al. (2011) reported that the exposure of pea seedlings to 10 µM IAA plus Mn (50-200 µM) increased the reduced of ascorbate and reduced glutathione, two ROS scavengers. In addition, long-term exposure to Cu induced nitric oxide (NO) generation in *Arabidopsis thaliana* (Brassicaceae), which has been associated with ROS scavenging and also with protein nitration Kolbert et al. (2012; Saxena and Shekhawat (2013). Moreover, a previous report indicates that IAA at low concentration increased protein content in *Catharanthus roseus* (Apocynaceae). G. Don (Muthulakshmi and Pandiyarajan (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 et al. 2011), increased the protein nitration in green pea seeds. More studies are needed in order to clarify the hypothesis.

Element accumulation in seeds

Concentrations of elements affected by treatments in seeds are shown in **Table 12.** As seen in this table, the combination of IAA at 100 µM with both nCuO at 100 mg/kg and bCuO at 50 mg/kg increased seed Fe by 141%,with respect to the IAA control. However, there was no difference compared with the absolute control, which suggests that the IAA, *per se*, had an effect. Conversely, IAA at 100 µM plus nCuO at 50 mg/kg reduced seed B by 37%, compared to IAA at 100 µM and about 83% compared with absolute control. In addition, IAA at 100 µM plus

nCuO at 50 mg/kg increased seed Al by 314% and 297% regarding the absolute control. Results with B in the current study are similar to the results reported by Le Van et al. (2016) in transgenic cotton; however, results with Fe are contrary to the results reported by the same authors. In addition, results in pea seed are contrary to the results reported by Choudhary et al. (2010) in radish seedlings, where Fe levels decrease under Cu exposure. Aluminum, a non-essential element, increase by 214% in plants exposed to 100 μM IAA with nCuO at 50 mg/kg, when compared to control. An increase of Al in lettuce exposed to nCu and nCuO, has also been reported by Trujillo-Reyes et al. (2014). The increase of seed Fe under nCuO plus IAA exposure could be related to the linkage of the Cu- and Fe-chelate reductase activities in the plasma membrane (Puig et al. 2007). Concerning B, Singh et al. (1990) reported an inverse relation between B and Cu 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, and increase in Al could represent a threat, since an excess in Al has been associated with Alzheimer's disease (Tomljenovic 2010).

Table 12 Elements affected on green pea seeds from plants grown for 90 days in soil amended with nCuO, bCuO, and CuCl2 (50 and 100 mg/kg of soil) and enhanced with IAA (0, 10, and 100μM). Data are average ± SE of four replicates. Comparisons were against controls, arrows indicate a decrease or increase in nutrient concentration.

Plant organ	Elements	Treatment (mg/kg soil)	Concentration (Cu mg/kg d wt tissue)	%	
Seeds	Fe	Control 3 (100 μM IAA)	44.0 ± 5.1	100%	
		100 μM IAA + nCuO 100 mg/kg	62.3 ± 5.1	141%	↑
		100 μM IAA + bCuO 50 mg/kg	61.9 ± 5.1	141%	↑
	B	Control 3 (100 μM IAA)	3.8 ± 0.6	100%	
		100 μM IAA + nCuO 50 mg/kg	1.4 ± 0.7	37%	↓
	Al	Control 3 (100 μM IAA)	4.2 ± 1.4	100%	
		100 μM IAA + nCuO 50 mg/kg	13.5 ± 1.2	314%	↑

Summary

Results of this study suggest nCuO 50 mg/kg and bCuO at 50 mg/kg alone decrease the green pea seed production. The lowest amount of seeds and lowest weight lay in treatments with 10 μM, when averaged over the whole Cu treatments. There were no significant differences found in the Cu translocation from plant organs to seeds. But 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 seeds. In addition, IAA at 10 μM increased Fe in treatments with 100 μM IAA + nCuO 100 mg/kg and, 100 μM IAA + bCuO 50 mg/kg and Al in treatment with 100 μM IAA + nCuO 50 mg/kg, but a decrease of B in treatment 100 μM IAA + nCuO 50 mg/kg.

Chapter 4: General Conclusions

The impact of CuO nanoparticles in crop plants is not yet well understood. Moreover, to the author's knowledge, this is the first study about the effects of nCuO under excess of exogenous auxins such as IAA. The objectives of this study were to evaluate the physiological and biochemical changes operated in green pea plants exposed to nCuO and two concentrations of IAA. To discriminate effects of the particle size and ionic copper, experiments included nCuO (10-100 nm), bCuO (100-10,000 nm), and $CuCl_2$. Plants were monitored through the full life cycle and analyzed at pod formation stage, where the maximum of photosynthates occurs, and at physiological maturity. The main factor analysis showed that IAA at 100 μM (averaged over all Cu treatments) increased Cu concentration in tissues and in pods with 10 μM IAA. Whereas, in seeds it decreased with the addition of 100 μM IAA. The average of the other main, Cu compounds, showed that nCuO did not affect biomass production; however, stem biomass increased in treatments with bCuO at 100 mg/kg, compared with controls. In general, the main effects of both IAA, specifically at 10 μM, and Cu compounds, specifically $CuCl_2$ at 50 an 100 mg/kg, showed a decrease in total chlorophyll chlo-*a*, chlo-*b,* and carotenoid. On the other hand, the CAT activity increased by 74.5% with the increment of 10 μM IAA. All the treatments affect the concentration of micro- and macroelements in tissues. In general, the highest Cu treatment concentration (100 mg/kg) increased Cu in roots. $CuCl_2$ and both concentration, plus IAA at 10 μM, increased Ca in roots. In stem, concentrations of B, Cu, Mo, and S increased with several combination treatments. However, Mn, Mg, and Ca, decreased mainly in plants exposed to bCuO and nCuO. In leaves, there was an increase in B, Cu, Ca, Mg, and S, while there was a decrease in Fe. Finally, nCuO at 50 mg/kg increased pod Fe and Ni; bCuO 100 mg/kg also increased pod Ni, while $CuCl_2$ at the two concentrations increased pod B. This study has shown that the

interaction between nCuO x IAA has several agronomical, physiological, and biochemical effects in green pea plants. The addition of the main factor IAA at 10 μM reduced the number of seeds, but it also increased the seed protein content. Suggesting a reduction in pea production with a fortification of seed nutritional quality. Overall, this study has shown that more research is needed in order to have a thoroughly understanding of the effects of nCuO in crops plants, under an excess of exogenous phytohormones.

References

CHAPTER 1

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Appendix

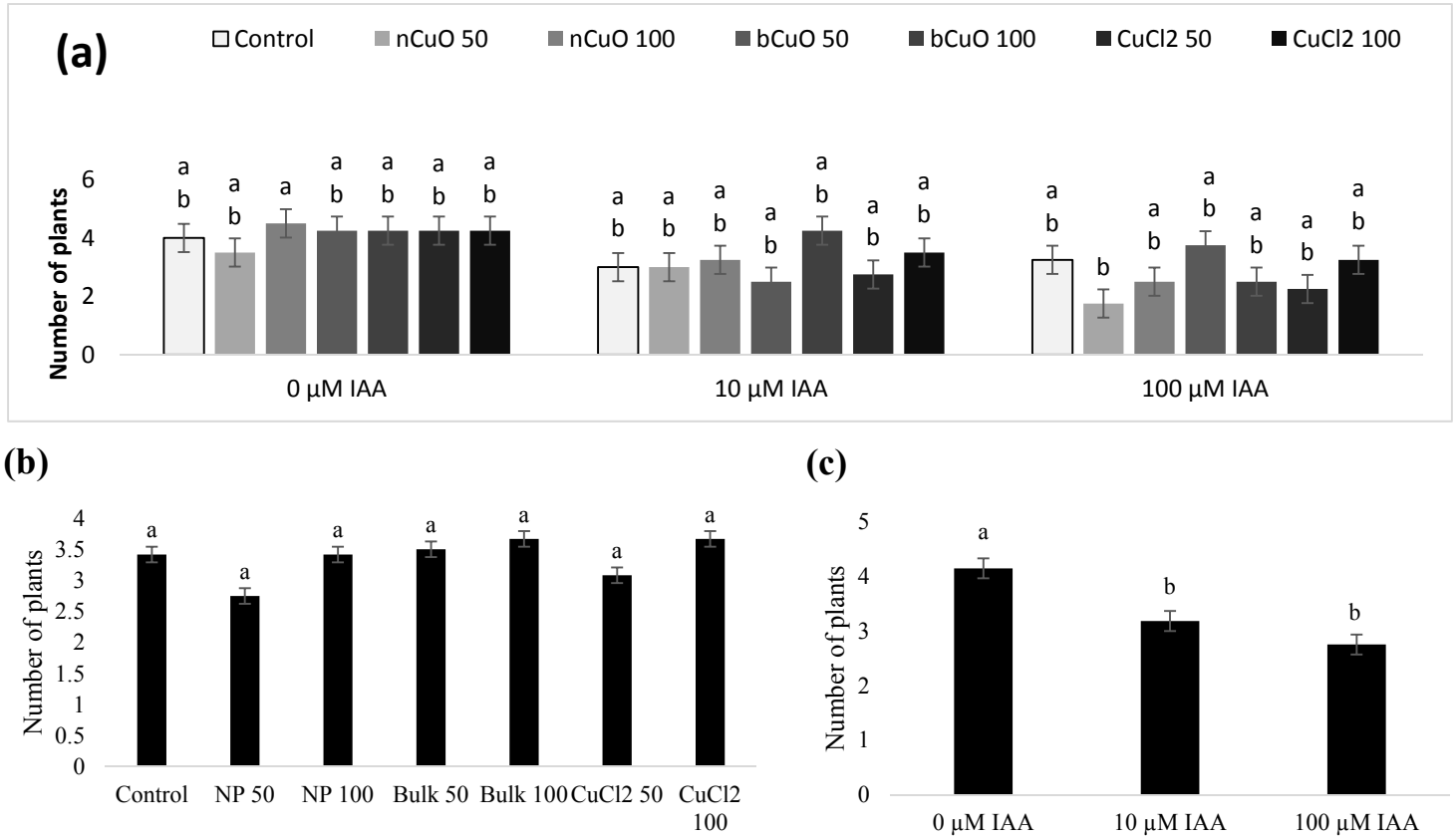

Figure S1. Survival of green pea plants at 45 days of growth in soil amended with nCuO, bCuO, and CuCl2 (50 and 100 mg/kg of soil) and enhanced with IAA (0, 10, and 100µM) (a) Number of plants per treatment. (b) Statistical differences in number of plants, when averaged by Cu compound treatments. (c) Statistical differences in number of plants when averaged by IAA (0, 10, 100). Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

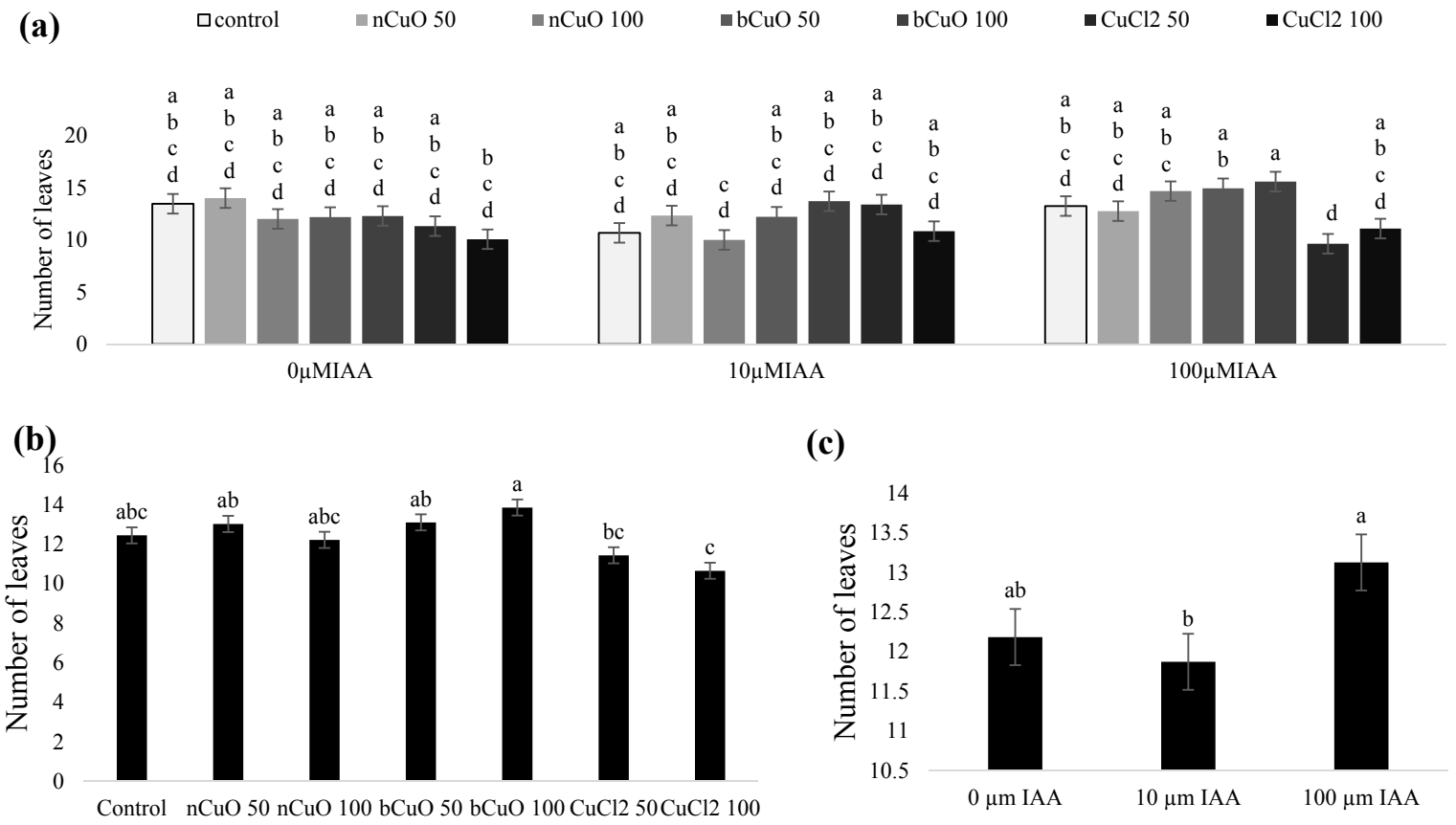

Figure S2 Number of leaves in green pea plants at 45 days of growth in soil amended with nCuO, bCuO, and CuCl2 (50 and 100 mg/kg of soil) and enhanced with IAA (0, 10, and 100µM) (a) Number of leaves per treatment. (b) Statistical differences in number of leaves when averaged by Cu compound treatments. (c) Statistical differences in number of leaves when averaged by IAA (0, 10, 100). Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$)

Table S1 Green pea plant organ length grouped by Cu compound levels (nCuO, bCuO, and CuCl2) at different levels and enhanced with IAA. Results are means ± SE. Letters indicate statistical differences ($p \leq 0.05$).

Cu Compound levels	Green pea plant organ length (cm) by Cu compounds		
	Stems (alone)	Roots (alone)	Shoot system
Control (0)	17.16 ± 1.13 ab	21.94 ± 1.40 bc	20.33 ± 1.19 a
nCuO 50	19.21 ± 1.13 a	26.52 ± 1.40 ab	22.05 ± 1.19 a
nCuO 100	15.81 ± 1.13 ab	29.07 ± 1.40 a	18.71 ± 1.19 a
bCuO 50	16.01 ± 1.13 ab	24.33 ± 1.40 abc	18.39 ± 1.19 a
bCuO 100	16.71 ± 1.13 ab	22.61 ± 1.40 bc	20.58 ± 1.19 a
CuCl2 50	14.75 ± 1.13 ab	23.00 ± 1.40 bc	18.26 ± 1.19 a
CuCl2 100	13.61 ± 1.13 b	18.73 ± 1.40 c	16.96 ± 1.19 a

Table S2 Green pea plant organ length grouped by IAA (μM) concentrations in green pea organs grown in soil amended with different Cu compounds (nCuO, bCuO, and CuCl2) at different levels and enhanced with IAA. Results are means ± SE. Letters indicate statistical differences ($p \leq 0.05$).

IAA concentration	Green pea plant organ length (cm) by IAA concentrations		
	Stems (alone)	Roots (alone)	Shoot system
0 μM IAA	16.88 ± 0.742 a	23.9 ± 0.916 a	19.7 ± 0.78 a
10 μM IAA	16.29 ± 0.742 a	22.2 ± 0.916 a	19.5 ± 0.78 a
100 μM IAA	15.38 ± 0.742 a	25.2 ± 0.916 a	18.8 ± 0.78 a

Table S 3

Elemental concentrations in soil from all the different treatments: 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE, letters indicate statistical differences within different concentrations and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments			Micronutrients				
ID	IAA (µM)	Cu Compounds (mg/kg of soil)	Cu	Fe	Mn	Zn	Ni
1	0	0	16.1 ± 8.3 h	16345.4 ± 549 ab	419.8 ± 15.7 a	51.7 ± 1.7 a	13.1 ± 0.46 a
2		n50	59.1 ± 8.3 defgh*	16002.5 ± 549 ab	415.0 ± 15.7 a	50.9 ± 1.7 a	13.0 ± 0.46 a
3		n100	98.6 ± 8.3 abcde*	16103.7 ± 549 ab	421.8 ± 15.7 a	51.1 ± 1.7 a	12.8 ± 0.46 a
4		b50	56.3 ± 8.3 efgh*	16407.0 ± 549 ab	421.5 ± 15.7 a	51.4 ± 1.7 a	12.9 ± 0.46 a
5		b100	93.8 ± 8.3 abcdef*	16736.0 ± 549 ab	430.5 ± 15.7 a	52.2 ± 1.7 a	13.5 ± 0.46 a
6		CuCl2 50	53.0 ± 8.3 fgh*	15968.5 ± 549 ab	409.3 ± 15.7 a	50.7 ± 1.7 a	12.9 ± 0.46 a
7		CuCl2 100	107.8 ± 8.3 abc*	16212.4 ± 549 ab	418.9 ± 15.7 a	50.6 ± 1.7 a	13.0 ± 0.46 a
8	10	0	16.2 ± 8.3 h	16156.5 ± 549 ab	417.6 ± 15.7 a	49.7 ± 1.7 a	12.9 ± 0.46 a
9		n50	51.8 ± 8.3 fgh	16016.7 ± 549 ab	413.0 ± 15.7 a	49.2 ± 1.7 a	12.7 ± 0.46 a
10		n100	102.9 ± 8.3 abcd*	16019.4 ± 549 ab	414.5 ± 15.7 a	49.5 ± 1.7 a	12.8 ± 0.46 a
11		b50	43.0 ± 8.3 gh	15767.6 ± 549 ab	411.4 ± 15.7 a	49.0 ± 1.7 a	12.6 ± 0.46 a
12		b100	91.1 ± 8.3 abcdef*	16113.4 ± 549 ab	416.9 ± 15.7 a	50.1 ± 1.7 a	13.1 ± 0.46 a
13		CuCl2 50	66.8 ± 8.3 cdefg*	16861.7 ± 549 a	442.3 ± 15.7 a	52.3 ± 1.7 a	13.7 ± 0.46 a
14		CuCl2 100	105.9 ± 8.3 abc*	16274.9 ± 549 ab	428.8 ± 15.7 a	50.0 ± 1.7 a	13.5 ± 0.46 a
15	100	0	17.2 ± 8.3 h	16130.4 ± 549 ab	424.6 ± 15.7 a	50.4 ± 1.7 a	13.3 ± 0.46 a
16		n50	81.6 ± 8.3 bcdefg*	16683.7 ± 549 ab	455.8 ± 15.7 a	51.7 ± 1.7 a	14.0 ± 0.46 a
17		n100	128.6 ± 8.3 a*	14228.5 ± 549 ab	422.2 ± 15.7 a	44.9 ± 1.7 a	12.1 ± 0.46 a
18		b50	75.1 ± 8.3 bcdefg*	15260.9 ± 549 ab	459.1 ± 15.7 a	48.7 ± 1.7 a	13.0 ± 0.46 a
19		b100	114.0 ± 8.3 ab*	15909.4 ± 549 ab	467.2 ± 15.7 a	50.1 ± 1.7 a	13.7 ± 0.46 a

20		CuCl2 50	58.8 ± 8.3 defgh	15760.6 ± 549 ab	452.8 ± 15.7 a	49.4 ± 1.7 a	13.1 ± 0.46 a
21		CuCl2 100	99.7 ± 8.3 abcde*	13803.8 ± 549 b	396.3 ± 15.7 a	43.9 ± 1.7 a	11.6 ± 0.46 a

Treatments			Macronutrients				
ID	IAA (µM)	Cu Compounds	P	K	Ca	Mg	S
1	0	0	730 ± 26.5 a	4981 ± 166.1 ab	27441 ± 1002.9 bc	7115 ± 264.7 ab	434 ± 22.8 a
2		n50	698 ± 26.5 a	4713 ± 166.1 abcd	26875 ± 1002.9 bc	6988 ± 264.7 ab	338 ± 22.8 abc*
3		n100	709 ± 26.5 a	4694 ± 166.1 abcd	27570 ± 1002.9 bc	6994 ± 264.7 ab	377 ± 22.8 abc
4		b50	702 ± 26.5 a	5123 ± 166.1 a	27178 ± 1002.9 bc	7284 ± 264.7 ab	325 ± 22.8 abc*
5		b100	693 ± 26.5 a	5186 ± 166.1 a	27639 ± 1002.9 bc	7399 ± 264.7 ab	320 ± 22.8 abc*
6		CuCl2 50	693 ± 26.5 a	4728 ± 166.1 abcd	26516 ± 1002.9 c	6949 ± 264.7 ab	386 ± 22.8 abc
7		CuCl2 100	695 ± 26.5 a	4956 ± 166.1 abc	26663 ± 1002.9 bc	7251 ± 264.7 ab	314 ± 22.8 abc*
8	10	0	719 ± 26.5 a	4984 ± 166.1 ab	26744 ± 1002.9 bc	7211 ± 264.7 ab	324 ± 22.8 abc
9		n50	706 ± 26.5 a	4992 ± 166.1 ab	26292 ± 1002.9 c	7168 ± 264.7 ab	321 ± 22.8 abc
10		n100	680 ± 26.5 a*	4984 ± 166.1 ab	26577 ± 1002.9 c	7230 ± 264.7 ab	321 ± 22.8 abc
11		b50	677 ± 26.5 a*	4762 ± 166.1 abc	27102 ± 1002.9 bc	7113 ± 264.7 ab	351 ± 22.8 abc
12		b100	676 ± 26.5 a*	4931 ± 166.1 abc	26789 ± 1002.9 bc	7195 ± 264.7 ab	299 ± 22.8 bc
13		CuCl2 50	723 ± 26.5 a	5218 ± 166.1 a	28947 ± 1002.9 abc	7727 ± 264.7 a	403 ± 22.8 ab
14		CuCl2 100	682 ± 26.5 a*	4813 ± 166.1 abc	27816 ± 1002.9 bc	7356 ± 264.7 ab	369 ± 22.8 abc
15	100	0	708 ± 26.5 a	4878 ± 166.1 abc	27367 ± 1002.9 bc	7385 ± 264.7 ab	281 ± 22.8 bc
16		n50	715 ± 26.5 a	4849 ± 166.1 abc	30222 ± 1002.9 abc	7785 ± 264.7 a	314 ± 22.8 abc
17		n100	632 ± 26.5 a	3799 ± 166.1 e	29511 ± 1002.9 abc	6552 ± 264.7 ab	280 ± 22.8 c
18		b50	720 ± 26.5 a	4063 ± 166.1 cde	32044 ± 1002.9 ab	7168 ± 264.7 ab	348 ± 22.8 abc
19		b100	741 ± 26.5 a	4211 ± 166.1 bcde	33256 ± 1002.9 a	7477 ± 264.7 ab	291 ± 22.8 bc
20		CuCl2 50	761 ± 26.5 a	4448 ± 166.1 abcde	30837 ± 1002.9 abc	7196 ± 264.7 ab	383 ± 22.8 abc
21		CuCl2 100	632 ± 26.5 a	3847 ± 166.1 de	27530 ± 1002.9 bc	6292 ± 264.7 b	299 ± 22.8 bc

Treatments			Other Metals			
ID	IAA (µM)	Cu Compounds	Al	Cr	Pb	Cd

1	0	0	19926 ± 614.4 a	17.5 ± 0.5 ab	18.8 ± 0.8 a	0.4 ± 0.09 a
2		n50	18565 ± 614.4 a	16.7 ± 0.5 abc	18.3 ± 0.8 a	0.2 ± 0.09 a
3		n100	18146 ± 614.4 ab	16.5 ± 0.5 abc	18.4 ± 0.8 a	0.4 ± 0.09 a
4		b50	20453 ± 614.4 a	17.7 ± 0.5 ab	19.3 ± 0.8 a	0.4 ± 0.09 a
5		b100	20519 ± 614.4 a	17.8 ± 0.5 ab	19.2 ± 0.8 a	0.2 ± 0.09 a
6		CuCl2 50	19083 ± 614.4 a	17.0 ± 0.5 abc	18.3 ± 0.8 a	0.3 ± 0.09 a
7		CuCl2 100	20133 ± 614.4 a	17.1 ± 0.5 abc	18.1 ± 0.8 a	0.2 ± 0.09 a
8	10	0	19829 ± 614.4 a	17.1 ± 0.5 abc	18.0 ± 0.8 a	0.2 ± 0.09 a
9		n50	20169 ± 614.4 a	17.1 ± 0.5 abc	18.7 ± 0.8 a	0.3 ± 0.09 a
10		n100	19893 ± 614.4 a	17.0 ± 0.5 abc	19.3 ± 0.8 a	0.4 ± 0.09 a
11		b50	19922 ± 614.4 a	16.8 ± 0.5 abc	18.4 ± 0.8 a	0.1 ± 0.09 a
12		b100	19844 ± 614.4 a	17.0 ± 0.5 abc	18.8 ± 0.8 a	0.3 ± 0.09 a
13		CuCl2 50	21049 ± 614.4 a	18.2 ± 0.5 a	19.6 ± 0.8 a	0.1 ± 0.09 a
14		CuCl2 100	20042 ± 614.4 a	17.2 ± 0.5 abc	19.0 ± 0.8 a	0.3 ± 0.09 a
15	100	0	19271 ± 614.4 a	16.9 ± 0.5 abc	18.9 ± 0.8 a	0.2 ± 0.09 a
16		n50	20628 ± 614.4 a	17.7 ± 0.5 ab	19.0 ± 0.8 a	0.3 ± 0.09 a
17		n100	13048 ± 614.4 c*	13.3 ± 0.5 d	18.9 ± 0.8 a	0.4 ± 0.09 a
18		b50	14234 ± 614.4 c*	14.4 ± 0.5 cd	20.6 ± 0.8 a	0.4 ± 0.09 a
19		b100	14964 ± 614.4 bc*	15.2 ± 0.5 bcd	19.7 ± 0.8 a	0.6 ± 0.09 a
20		CuCl2 50	14035 ± 614.4 c*	14.5 ± 0.5 cd	18.7 ± 0.8 a	0.5 ± 0.09 a
21		CuCl2 100	12329 ± 614.4 c*	12.8 ± 0.5 d*	16.8 ± 0.8 a	0.2 ± 0.09 a

Table S 4

Iron concentrations [Fe] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments			[Fe] mg/kg in different plant tissues			
ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	11.9 ± 5.1 b	116.2 ± 10.6 abc	54.2 ± 6.2 ab	870.9 ± 138.6 a
2		n50	42.6 ± 5.1 a*	115.7 ± 10.6 abc	37.1 ± 7.1 b	1014.5 ± 138.6 a
3		n100	16.2 ± 5.1 ab	125.2 ± 10.6 abc	50.7 ± 6.2 ab	998.0 ± 160.1 a
4		b50	32.5 ± 5.1 ab	122.2 ± 10.6 abc	52.5 ± 6.2 ab	949.2 ± 138.6 a
5		b100	14.4 ± 5.1 b	114.5 ± 10.6 abcd	53.0 ± 6.2 ab	1225.1 ± 138.6 a
6		CuCl2 50	14.2 ± 5.1 b	92.7 ± 10.6 abcd	52.3 ± 6.2 ab	903.0 ± 138.6 a
7		CuCl2 100	11.0 ± 5.1 b	101.8 ± 10.6 abcd	53.0 ± 6.2 ab	814.8 ± 138.6 a
8	10	0	18.1 ± 5.1 ab	124.7 ± 10.6 abc	61.1 ± 6.2 ab	936.8 ± 138.6 a
9		n50	14.7 ± 5.1 b	103.5 ± 10.6 abcd	62.3 ± 6.2 ab	1001.6 ± 138.6 a
10		n100	28.6 ± 5.1 ab	109.0 ± 10.6 abcd	54.7 ± 6.2 ab	716.1 ± 138.6 a
11		b50	20.3 ± 5.1 ab	111.1 ± 10.6 abcd	60.5 ± 6.2 ab	824.0 ± 138.6 a
12		b100	35.2 ± 5.9 ab	118.0 ± 10.6 abc	48.9 ± 6.2 ab	1108.0 ± 138.6 a
13		CuCl2 50	14.1 ± 5.1 b	87.2 ± 10.6 bcd	58.3 ± 6.2 ab	980.7 ± 138.6 a
14		CuCl2 100	31.1 ± 5.1 ab	143.4 ± 10.6 a	48.5 ± 6.2 ab	1003.2 ± 138.6 a
15	100	0	8.3 ± 5.1 b	117.5 ± 10.6 abc	64.6 ± 6.2 ab	858.0 ± 138.6 a
16		n50	16.1 ± 5.9 ab	127.7 ± 10.6 ab	82.0 ± 6.2 a	914.6 ± 138.6 a
17		n100	7.6 ± 5.1 b	123.2 ± 10.6 abc	41.2 ± 7.1 b	1049.6 ± 138.6 a
18		b50	26.4 ± 5.1 ab	117.5 ± 10.6 abc	61.8 ± 7.1 ab	972.1 ± 138.6 a
19		b100	10.1 ± 5.1 b	124.7 ± 10.6 abc	51.8 ± 6.2 ab	1240.5 ± 138.6 a

20		CuCl2 50	7.8 ± 5.9 b	69.6 ± 10.6 cd*	66.4 ± 6.2 ab	1133.9 ± 138.6 a
21		CuCl2 100	16.6 ± 5.9 ab	58.7 ± 10.6 d*	48.8 ± 6.2 ab	1042.3 ± 138.6 a

± represent standard error.

Table S 5

Manganese concentrations [Mn] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments			[Mn] mg/kg in different plant tissues			
ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	25.1 ± 3.1 a	102.8 ± 9.5 abcd	14.0 ± 0.6 abc	34.6 ± 4.7 ab
2		n50	26.9 ± 3.1 a	89.6 ± 9.5 bcd	12.7 ± 0.6 c	37.6 ± 4.7 ab
3		n100	21.1 ± 3.1 a	110.0 ± 9.5 abcd	14.2 ± 0.6 abc	37.5 ± 4.7 ab
4		b50	28.9 ± 3.1 a	113.3 ± 9.5 abcd	14.6 ± 0.6 abc	35.7 ± 4.7 ab
5		b100	21.6 ± 3.1 a	108.7 ± 9.5 abcd	12.8 ± 0.6 bc	35.5 ± 4.7 ab
6		CuCl2 50	28.3 ± 3.1 a	79.2 ± 9.5 d	13.1 ± 0.6 bc	39.0 ± 4.7 ab
7		CuCl2 100	31.7 ± 3.1 a	81.8 ± 9.5 cd	13.9 ± 0.6 abc	35.3 ± 4.7 ab
8	10	0	25.2 ± 3.1 a	109.8 ± 9.5 abcd	16.1 ± 0.6 abc	42.8 ± 4.7 ab
9		n50	23.9 ± 3.1 a	98.7 ± 9.5 abcd	16.3 ± 0.8 ab	42.9 ± 4.7 ab
10		n100	28.2 ± 3.1 a	89.6 ± 9.5 bcd	13.1 ± 0.6 bc*	30.6 ± 4.7 b
11		b50	24.2 ± 3.1 a	90.9 ± 9.5 bcd	14.2 ± 0.6 abc	37.2 ± 4.7 ab
12		b100	29.5 ± 3.5 a	108.2 ± 9.5 abcd	13.4 ± 0.6 abc	42.2 ± 4.7 ab

13		CuCl2 50	24.3 ± 3.1 a	99.8 ± 9.5 abcd	14.6 ± 0.6 abc	36.7 ± 4.7 ab
14		CuCl2 100	23.2 ± 3.5 a	130.7 ± 9.5 abc	12.6 ± 0.6 c*	39.4 ± 4.7 ab
15	100	0	27.3 ± 3.1 a	139.4 ± 9.5 ab	15.9 ± 0.6 abc	39.2 ± 4.7 ab
16		n50	24.4 ± 3.5 a	146.7 ± 9.5 a	16.9 ± 0.8 a	41.6 ± 4.7 ab
17		n100	19.6 ± 3.1 a	127.8 ± 9.5 abcd	14.7 ± 0.6 abc	39.8 ± 4.7 ab
18		b50	26.9 ± 3.1 a	146.2 ± 9.5 a	15.9 ± 0.8 abc	39.7 ± 4.7 ab
19		b100	20.5 ± 3.1 a	120.3 ± 9.5 abcd	15.2 ± 0.6 abc	42.6 ± 4.7 ab
20		CuCl2 50	20.2 ± 3.1 a	118.5 ± 9.5 abcd	14.1 ± 0.6 abc	59.1 ± 4.7 a
21		CuCl2 100	20.7 ± 3.5 a	89.7 ± 9.5 bcd	13.9 ± 0.8 abc	42.2 ± 4.7 ab

± represent standard error.

Table S 6

Zinc concentrations [Zn] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments			[Zn] mg/kg in different plant tissues			
ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	11.1 ±5.7 a	25.4 ± 3.9 cd	37.8 ± 6.4 bcd	34.0 ± 4.8 b
2		n50	17.3 ±5.7 a	21.3 ± 3.9 cd	39.6 ± 5.6 abcd	37.1 ± 4.8 b
3		n100	6.3 ±5.7 a	24.4 ± 3.9 cd	35.0 ± 5.6 bcd	30.4 ± 4.8 b
4		b50	27.1 ±5.7 a	30.5 ± 4.5 bcd	46.5 ± 5.6 abcd	37.2 ± 4.8 b
5		b100	11.8 ±5.7 a	26.8 ± 3.9 cd	45.4 ± 5.6 abcd	34.5 ± 5.6 b

6		CuCl2 50	19.2 ±5.7 a	23.7 ± 3.9 cd	30.0 ± 5.6 bcd	34.5 ± 4.8 b
7		CuCl2 100	15.6 ±6.6 a	19.9 ± 3.9 cd	22.4 ± 5.6 d	30.4 ± 4.8 b
8	10	0	27.8 ±5.7 a	27.4 ± 3.9 cd	43.7 ± 5.6 abcd	42.1 ± 4.8 b
9		n50	22.3 ±5.7 a	26.2 ± 3.9 cd	38.5 ± 5.6 bcd	38.2 ± 4.8 b
10		n100	25.6 ±5.7 a	21.8 ± 3.9 cd	24.3 ± 5.6 cd	29.4 ± 4.8 b
11		b50	24.0 ±5.7 a	30.5 ± 3.9 bcd	38.0 ± 5.6 bcd	43.6 ± 4.8 b
12		b100	35.8 ±6.6 a	25.9 ± 3.9 cd	30.7 ± 5.6 bcd	41.2 ± 4.8 b
13		CuCl2 50	17.3 ±6.6 a	37.2 ± 3.9 bcd	47.8 ± 5.6 abcd	48.6 ± 4.8 b
14		CuCl2 100	36.4 ±5.7 a	33.5 ± 3.9 bcd	52.7 ± 5.6 abc	49.0 ± 4.8 b
15	100	0	12.5 ±5.7 a	37.0 ± 3.9 bcd	52.7 ± 5.6 abc	47.7 ± 4.8 b
16		n50	7.3 ±6.6 a	34.3 ± 3.9 bcd	38.9 ± 5.6 bcd	39.5 ± 4.8 b
17		n100	7.1 ±5.7 a	38.8 ± 3.9 abc	42.8 ± 5.6 abcd	55.2 ± 5.6 ab
18		b50	18.4 ±5.7 a	49.2 ± 3.9 ab	55.0 ± 5.6 ab	51.0 ± 4.8 b
19		b100	10.0 ±5.7 a	58.1 ± 3.9 a	69.0 ± 5.6 a	55.1 ± 5.6 ab
20		CuCl2 50	13.2 ±5.7 a	27.0 ± 3.9 cd	36.4 ± 5.6 bcd	80.9 ± 4.8 a
21		CuCl2 100	11.7 ±6.6 a	17.2 ± 3.9 d	42.7 ± 5.6 abcd	54.5 ± 4.8 b

± represent standard error.

Table S 7

Boron concentrations [B] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments	[B] mg/kg in different plant tissues

ID	IAA (μM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems
1	0	0	25.9 ± 2.9 a	2.0 ± 6.9 f	5.8 ± 1.2 abcd
2		n50	28.1 ± 2.9 a	8.5 ± 6.9 ef	5.6 ± 1.2 abcd
3		n100	31.7 ± 2.9 a	16.2 ± 6.0 ef	6.2 ± 1.2 abcd
4		b50	30.0 ± 2.9 a	14.3 ± 6.0 ef	3.8 ± 1.4 cd
5		b100	27.7 ± 2.9 a	10.2 ± 6.0 ef	4.1 ± 1.4 bcd
6		CuCl2 50	38.2 ± 2.9 a*	11.8 ± 6.0 ef	5.8 ± 1.4 abcd
7		CuCl2 100	38.0 ± 2.9 a*	19.7 ± 6.0 ef*	2.7 ± 1.2 d
8	10	0	37.6 ± 2.9 a	21.8 ± 6.0 ef	N/A ± N/A
9		n50	39.0 ± 2.9 a	16.0 ± 6.0 ef	3.8 ± 1.2 cd
10		n100	37.7 ± 2.9 a	13.9 ± 6.0 ef	4.4 ± 1.4 bcd
11		b50	38.3 ± 2.9 a	21.5 ± 6.0 ef	N/A ± N/A
12		b100	37.4 ± 3.4 a	19.7 ± 6.0 ef	5.7 ± 1.2 abcd
13		CuCl2 50	41.5 ± 2.9 a	21.3 ± 6.0 ef	7.0 ± 1.2 abcd
14		CuCl2 100	37.9 ± 2.9 a	40.0 ± 6.0 de	6.7 ± 1.2 abcd
15	100	0	28.4 ± 2.9 a	28.8 ± 6.0 def	3.2 ± 1.2 d
16		n50	29.7 ± 3.4 a	78.8 ± 6.0 bc*	4.2 ± 1.4 bcd
17		n100	28.6 ± 2.9 a	92.2 ± 6.0 ab*	8.4 ± 1.2 abcd
18		b50	26.6 ± 2.9 a	96.2 ± 6.0 ab*	11.7 ± 1.2 a*
19		b100	27.8 ± 2.9 a	111.7 ± 6.0 a*	10.3 ± 1.2 abc*
20		CuCl2 50	27.9 ± 2.9 a	30.4 ± 6.0 def	4.3 ± 1.2 bcd
21		CuCl2 100	27.8 ± 3.4 a	55.2 ± 6.0 cd	10.8 ± 1.2 ab*

± represent standard error.

Table S 8

Molybdenum concentrations [Mo] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments			[Mo] mg/kg in different plant tissues			
ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	5.2 ± 3.1 a	1.4 ± 1.5 b	31.5 ± 6.0 bcd	4.7 ± 1.8 bc
2		n50	9.2 ± 3.1 a	2.9 ± 1.3 b	34.7 ± 6.0 bcd	6.8 ± 1.8 abc
3		n100	4.3 ± 3.1 a	3.2 ± 1.3 b	43.4 ± 6.0 bcd	6.8 ± 1.8 abc
4		b50	16.9 ± 3.1 a	3.1 ± 1.5 b	39.4 ± 6.0 bcd	7.8 ± 1.8 abc
5		b100	8.4 ± 3.1 a	1.5 ± 1.5 b	39.1 ± 6.0 bcd	8.2 ± 1.8 abc
6		CuCl2 50	9.0 ± 3.1 a	N/A ± N/A	32.0 ± 6.0 bcd	2.9 ± 1.8 c
7		CuCl2 100	10.5 ± 3.1 a	N/A ± N/A	25.5 ± 6.0 d	4.7 ± 1.8 bc
8	10	0	9.0 ± 3.1 a	1.4 ± 1.5 b	36.5 ± 6.0 bcd	6.9 ± 1.8 abc
9		n50	4.6 ± 3.6 a	2.0 ± 1.5 b	38.9 ± 6.0 bcd	6.9 ± 1.8 abc
10		n100	15.1 ± 3.1 a	4.3 ± 1.3 ab	41.4 ± 6.0 bcd	8.8 ± 1.8 abc
11		b50	7.5 ± 3.1 a	5.9 ± 1.5 ab	42.4 ± 6.0 bcd	3.9 ± 2.1 bc
12		b100	12.1 ± 3.6 a	1.9 ± 1.5 b	39.7 ± 6.0 bcd	5.8 ± 1.8 abc
13		CuCl2 50	15.4 ± 3.1 a	1.1 ± 1.5 b	23.5 ± 6.0 d	7.0 ± 1.8 abc
14		CuCl2 100	8.6 ± 3.1 a	1.9 ± 1.5 b	30.5 ± 6.0 cd	6.7 ± 1.8 abc
15	100	0	5.7 ± 3.1 a	7.0 ± 1.5 ab	44.6 ± 6.0 bcd	7.2 ± 2.1 abc
16		n50	11.7 ± 3.6 a	11.0 ± 1.3 a	62.6 ± 6.0 ab	9.8 ± 1.8 abc
17		n100	12.4 ± 3.1 a	6.3 ± 1.3 ab	58.4 ± 6.0 abc	10.8 ± 1.8 abc
18		b50	14.3 ± 3.1 a	6.9 ± 1.3 ab	53.8 ± 6.0 abcd	11.2 ± 1.8 abc
19		b100	8.5 ± 3.1 a	7.6 ± 1.3 ab	82.5 ± 6.0 a*	12.6 ± 1.8 ab
20		CuCl2 50	10.3 ± 3.6 a	2.8 ± 1.3 b	34.8 ± 6.0 bcd	14.4 ± 1.8 a

21		CuCl2 100	5.7 ± 3.6 a	2.1 ± 1.3 b	33.4 ± 6.0 bcd	10.5 ± 1.8 abc

± represent standard error.

Table S 9

Nickel concentrations [Ni] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments			[Ni] mg/kg in different plant tissues			
ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	0.4 ± 0.2 a	1.3 ± 0.2 ab	1.4 ± 0.2 a	2.6 ± 0.3 b
2		n50	1.7 ± 0.2 a*	1.3 ± 0.2 ab	1.2 ± 0.2 a	2.8 ± 0.3 b
3		n100	0.7 ± 0.2 a	1.2 ± 0.2 ab	1.4 ± 0.2 a	3.4 ± 0.3 ab
4		b50	1.5 ± 0.2 a*	1.2 ± 0.2 ab	1.8 ± 0.2 a	3.3 ± 0.3 ab
5		b100	0.7 ± 0.2 a	1.3 ± 0.2 ab	1.6 ± 0.2 a	3.3 ± 0.3 ab
6		CuCl2 50	0.9 ± 0.2 a	1.0 ± 0.2 ab	1.2 ± 0.2 a	2.8 ± 0.3 b
7		CuCl2 100	0.6 ± 0.2 a	1.3 ± 0.2 ab	1.4 ± 0.2 a	3.0 ± 0.4 b
8	10	0	1.1 ± 0.2 a	1.2 ± 0.2 ab	1.1 ± 0.2 a	3.0 ± 0.3 b
9		n50	0.9 ± 0.2 a	1.3 ± 0.2 ab	1.8 ± 0.2 a	3.1 ± 0.3 b
10		n100	1.0 ± 0.2 a	1.5 ± 0.2 ab	1.6 ± 0.2 a	3.2 ± 0.3 ab
11		b50	1.3 ± 0.2 a	1.1 ± 0.2 ab	1.3 ± 0.2 a	3.1 ± 0.3 b
12		b100	1.5 ± 0.3 a	1.1 ± 0.2 ab	1.2 ± 0.2 a	3.0 ± 0.3 b
13		CuCl2 50	0.8 ± 0.2 a	1.4 ± 0.2 ab	1.1 ± 0.2 a	2.8 ± 0.3 b

14		CuCl2 100	1.6 ± 0.2 a	0.9 ± 0.2 ab	1.1 ± 0.2 a	2.7 ± 0.3 b
15	100	0	0.4 ± 0.2 a	1.2 ± 0.2 ab	1.0 ± 0.2 a	3.1 ± 0.3 b
16		n50	0.5 ± 0.3 a	1.6 ± 0.2 a	1.1 ± 0.2 a	2.7 ± 0.3 b
17		n100	0.5 ± 0.2 a	1.2 ± 0.2 ab	1.0 ± 0.2 a	2.8 ± 0.3 b
18		b50	0.7 ± 0.2 a	1.2 ± 0.2 ab	1.1 ± 0.2 a	2.6 ± 0.3 b
19		b100	0.4 ± 0.2 a	1.1 ± 0.2 ab	0.9 ± 0.2 a	2.5 ± 0.3 b
20		CuCl2 50	0.5 ± 0.2 a	1.2 ± 0.2 ab	1.0 ± 0.2 a	5.0 ± 0.3 a
21		CuCl2 100	0.5 ± 0.3 a	0.6 ± 0.2 b	1.0 ± 0.2 a	2.8 ± 0.4 b

± represent standard error.

Table S 10

Phosphorus concentrations [P] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments			[P] mg/kg in different plant tissues			
ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	256.4 ± 450.5 c	2460.2 ± 421.3 a	1705.9 ±275.7 ab	2047.5 ± 361.1 b
2		n50	1528.8 ± 450.5 abc	2631.2 ± 421.3 a	2073.0 ±275.7 ab	2370.7 ± 361.1 ab
3		n100	264.1 ± 450.5 c	2981.1 ± 421.3 a	1618.2 ±275.7 ab	2033.1 ± 361.1 b
4		b50	1559.5 ± 450.5 abc	2968.2 ± 421.3 a	1850.0 ±275.7 ab	2315.0 ± 361.1 b
5		b100	270.4 ± 520.2 c	2197.5 ± 421.3 a	1550.7 ±275.7 ab	2022.2 ± 361.1 b
6		CuCl2 50	427.2 ± 450.5 bc	2772.1 ± 421.3 a	1533.3 ±275.7 ab	2642.9 ± 361.1 ab

7		CuCl2 100	573.1 ± 450.5 bc	2333.6 ± 421.3 a	1165.5 ±275.7 ab	2246.2 ± 361.1 b
8	10	0	1102.8 ± 450.5 abc	3284.9 ± 421.3 a	2202.1 ±275.7 ab	2713.7 ± 361.1 ab
9		n50	459.4 ± 520.2 bc	3106.0 ± 421.3 a	1773.5 ±275.7 ab	2843.2 ± 361.1 ab
10		n100	1905.9 ± 450.5 abc	2434.7 ± 421.3 a	1294.7 ±275.7 ab	1800.7 ± 361.1 b
11		b50	1071.3 ± 450.5 abc	3078.2 ± 421.3 a	1649.0 ±275.7 ab	3450.0 ± 361.1 ab
12		b100	3437.8 ± 520.2 a	2841.0 ± 421.3 a	1046.0 ±275.7 b	2462.9 ± 361.1 ab
13		CuCl2 50	777.9 ± 450.5 bc	3681.9 ± 421.3 a	1882.8 ±275.7 ab	2864.2 ± 361.1 ab
14		CuCl2 100	2799.7 ± 450.5 ab	2787.2 ± 421.3 a	1818.7 ±275.7 ab	2531.3 ± 361.1 ab
15	100	0	322.1 ± 450.5 bc	3819.0 ± 421.3 a	1650.7 ±275.7 ab	2656.8 ± 361.1 ab
16		n50	348.8 ± 520.2 bc	3942.4 ± 421.3 a	1985.0 ±275.7 ab	2347.6 ± 361.1 ab
17		n100	254.5 ± 450.5 c	3600.1 ± 421.3 a	1954.1 ±275.7 ab	3206.9 ± 361.1 ab
18		b50	1642.9 ± 450.5 abc	3678.0 ± 421.3 a	2312.8 ±275.7 ab	3170.3 ± 361.1 ab
19		b100	328.3 ± 450.5 bc	3497.6 ± 421.3 a	2526.9 ±275.7 a	3194.4 ± 361.1 ab
20		CuCl2 50	313.1 ± 520.2 bc	2437.5 ± 421.3 a	1766.8 ±318.4 ab	4227.1 ± 361.1 a
21		CuCl2 100	1061.1 ± 520.2 abc	2016.1 ± 421.3 a	1383.3 ±275.7 ab	2719.3 ± 361.1 ab

± represent standard error.

Table S 11

Potassium concentrations [K] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments	[K] mg/kg in different plant tissues

ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	25311 ± 3260.4 a	421 ± 1524.8 a	36462 ± 2702.3 a	29177 ± 3000.0 ab
2		n50	23185 ± 3764.8 a	421 ± 1524.8 a	32392 ± 2702.3 a	28561 ± 3000.0 ab
3		n100	22173 ± 3260.4 a	421 ± 1524.8 a	34616 ± 2702.3 a	29680 ± 3000.0 ab
4		b50	27270 ± 3260.4 a	421 ± 1524.8 a	30520 ± 2702.3 a	29880 ± 3000.0 ab
5		b100	25804 ± 3260.4 a	421 ± 1524.8 a	33014 ± 2702.3 a	28110 ± 3000.0 ab
6		CuCl2 50	27238 ± 3260.4 a	421 ± 1524.8 a	32925 ± 2702.3 a	31811 ± 3000.0 ab
7		CuCl2 100	31000 ± 3260.4 a	421 ± 1524.8 a	27088 ± 2702.3 a	30927 ± 3000.0 ab
8	10	0	33820 ± 3260.4 a	421 ± 1524.8 a	32090 ± 2702.3 a	33307 ± 3000.0 ab
9		n50	27268 ± 3260.4 a	421 ± 1524.8 a	34593 ± 3120.4 a	34338 ± 3000.0 ab
10		n100	26336 ± 3260.4 a	421 ± 1524.8 a	26676 ± 2702.3 a	34236 ± 3000.0 ab
11		b50	29444 ± 3764.8 a	421 ± 1524.8 a	28358 ± 2702.3 a	34213 ± 3000.0 ab
12		b100	26512 ± 3764.8 a	421 ± 1524.8 a	33282 ± 2702.3 a	38306 ± 3000.0 ab
13		CuCl2 50	32260 ± 3260.4 a	421 ± 1524.8 a	27588 ± 2702.3 a	29247 ± 3000.0 ab
14		CuCl2 100	37702 ± 3260.4 a	421 ± 1524.8 a	28615 ± 2702.3 a	25097 ± 3000.0 ab
15	100	0	23567 ± 3260.4 a	421 ± 1524.8 a	31886 ± 2702.3 a	30828 ± 3000.0 ab
16		n50	26835 ± 3764.8 a	421 ± 1524.8 a	26885 ± 2702.3 a	29002 ± 3000.0 ab
17		n100	22082 ± 3260.4 a	421 ± 1524.8 a	29961 ± 2702.3 a	33413 ± 3000.0 ab
18		b50	25278 ± 3260.4 a	421 ± 1524.8 a	33033 ± 2702.3 a	34020 ± 3000.0 ab
19		b100	22459 ± 3260.4 a	421 ± 1524.8 a	30967 ± 2702.3 a	40085 ± 3000.0 a
20		CuCl2 50	25661 ± 3260.4 a	421 ± 1524.8 a	22870 ± 2702.3 a	38995 ± 3000.0 ab
21		CuCl2 100	27518 ± 3764.8 a	421 ± 1524.8 a	23306 ± 2702.3 a	23802 ± 3000.0 b

± represent standard error.

Table S 12

Calcium concentrations [Ca] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments			[Ca] mg/kg in different plant tissues			
ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	16699 ± 1513.9 a	35226 ± 3235 cd	14671 ± 1082 bcde	10420 ± 2283 b
2		n50	16830 ± 1513.9 a	39347 ± 3235 bcd	12032 ± 1082 de	10479 ± 2636 b
3		n100	16992 ± 1513.9 a	44728 ± 3235 abcd	16096 ± 1082 bcde	9826 ± 2636 b
4		b50	17303 ± 1513.9 a	50860 ± 3235 abc*	17919 ± 1082 bc	9747 ± 2283 b
5		b100	16496 ± 1513.9 a	51215 ± 3235 abc*	16844 ± 1082 bcde	8998 ± 2636 b
6		CuCl2 50	18569 ± 1513.9 a	52008 ± 3235 abc*	19478 ± 1250 ab*	10505 ± 2283 b
7		CuCl2 100	19926 ± 1513.9 a	60660 ± 3235 a*	23920 ± 1082 a*	10601 ± 2283 b
8	10	0	17233 ± 1513.9 a	49385 ± 3235 abcd	19216 ± 1082 ab	12064 ± 2283 b
9		n50	19235 ± 1513.9 a	48529 ± 3735 abcd	19593 ± 1082 ab	14719 ± 2283 b
10		n100	19542 ± 1513.9 a	46468 ± 3235 abcd	17775 ± 1082 bcd	10648 ± 2283 b
11		b50	19002 ± 1513.9 a	46831 ± 3235 abcd	18325 ± 1082 ab	16823 ± 2283 b
12		b100	17420 ± 1748.1 a	49925 ± 3235 abcd	17218 ± 1082 bcd	16386 ± 2283 b
13		CuCl2 50	20796 ± 1513.9 a	60111 ± 3735 a	15950 ± 1082 bcde	17823 ± 2283 b*
14		CuCl2 100	21571 ± 1513.9 a	60793 ± 3235 a	14001 ± 1082 bcde	19559 ± 2283 ab*
15	100	0	19472 ± 1748.1 a	51861 ± 3235 abc	18121 ± 1082 b	16357 ± 2283 b
16		n50	16919 ± 1748.1 a	60804 ± 3235 a	17845 ± 1082 bc	16286 ± 2283 b
17		n100	16884 ± 1513.9 a	48747 ± 3235 abcd	12328 ± 1082 cde*	17714 ± 2283 b
18		b50	14456 ± 1513.9 a	46277 ± 3235 abcd	14126 ± 1082 bcde	20727 ± 2283 ab
19		b100	15763 ± 1513.9 a	48575 ± 3235 abcd	11389 ± 1082 e*	18559 ± 2283 ab
20		CuCl2 50	16050 ± 1513.9 a	55191 ± 3235 ab	15894 ± 1082 bcde	30748 ± 2283 a

21		CuCl2 100	17159 ± 1748.1 a	33013 ± 3235 d*	16040 ± 1082 bcde	18854 ± 2283 ab

± represent standard error.

Table S 13

Magnesium concentrations [Mg] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments			[Mg] mg/kg in different plant tissues			
ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	3488 ± 281 cd	4506 ± 380 c	5275 ± 371 abc	7322 ± 1070 a
2		n50	3795 ± 281 abcd	5185 ± 329 bc	4395 ± 371 bc	6032 ± 1070 a
3		n100	3682 ± 281 bcd	5690 ± 329 abc	6104 ± 371 ab	7390 ± 1070 a
4		b50	3782 ± 281 abcd	6041 ± 329 abc*	6161 ± 371 ab	6163 ± 1070 a
5		b100	3926 ± 281 abcd	6125 ± 329 abc*	6117 ± 371 ab	6861 ± 1070 a
6		CuCl2 50	4086 ± 281 abcd	6236 ± 329 abc*	5593 ± 371 abc	7552 ± 1070 a
7		CuCl2 100	4240 ± 281 abcd	6830 ± 329 ab*	6260 ± 371 ab	9053 ± 1070 a
8	10	0	4819 ± 281 abc	5922 ± 329 abc	6307 ± 371 ab	5733 ± 1070 a
9		n50	4834 ± 281 abc	5948 ± 329 abc	5594 ± 371 abc	6098 ± 1070 a
10		n100	4540 ± 281 abcd	4966 ± 329 c	6027 ± 371 ab	8753 ± 1070 a
11		b50	4563 ± 281 abcd	5368 ± 329 abc	5745 ± 371 ab	6157 ± 1070 a
12		b100	4393 ± 324 abcd	5709 ± 329 abc	5722 ± 371 abc	8468 ± 1070 a
13		CuCl2 50	5217 ± 281 a	5709 ± 329 abc	5304 ± 429 abc	6573 ± 1070 a

14		CuCl2 100	5181 ± 281 ab	6081 ± 329 abc	4636 ± 371 abc	6120 ± 1070 a
15	100	0	3875 ± 281 abcd	5684 ± 329 abc	5823 ± 371 ab	6438 ± 1070 a
16		n50	3490 ± 324 cd	7095 ± 329 a*	6572 ± 371 a	8790 ± 1070 a
17		n100	3940 ± 281 abcd	5460 ± 329 abc	3757 ± 371 c*	7484 ± 1070 a
18		b50	3420 ± 281 cd	5031 ± 329 c	4768 ± 371 abc	7780 ± 1070 a
19		b100	3807 ± 281 abcd	5567 ± 329 abc	3767 ± 371 c*	6239 ± 1070 a
20		CuCl2 50	3499 ± 281 cd	6007 ± 329 abc	5419 ± 371 abc	9374 ± 1070 a
21		CuCl2 100	3268 ± 324 d	4595 ± 329 c	4722 ± 371 abc	7140 ± 1070 a

± represent standard error.

Table S 14

Sulfur concentrations [S] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments			[S] mg/kg in different plant tissues			
ID	IAA (µM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	346 ± 436.9 b	2623 ± 747.9 d	3676 ± 596.2 de	5565 ± 638.2 b
2		n50	999 ± 436.9 ab	3593 ± 747.9 cd	3085 ± 596.2 e	5983 ± 638.2 b
3		n100	238 ± 436.9 b	4379 ± 747.9 bcd	4518 ± 596.2 bcde	6061 ± 737.0 b
4		b50	1355 ± 436.9 ab	4158 ± 747.9 cd	4125 ± 688.4 cde	6432 ± 737.0 ab
5		b100	781 ± 436.9 ab	4029 ± 747.9 cd	4569 ± 596.2 bcde	5532 ± 638.2 b
6		CuCl2 50	1015 ± 436.9 ab	8328 ± 747.9 ab*	7059 ± 596.2 abc*	6704 ± 638.2 ab

7		CuCl2 100	1445 ± 436.9 ab	9554 ± 747.9 a*	7998 ± 596.2 a*	6270 ± 638.2 b
8	10	0	1625 ± 436.9 ab	7314 ± 747.9 abc	6659 ± 596.2 abcd	7101 ± 638.2 ab
9		n50	923 ± 436.9 ab	6724 ± 747.9 abc	7405 ± 596.2 ab	7417 ± 638.2 ab
10		n100	1795 ± 436.9 ab	4830 ± 747.9 bcd	5593 ± 596.2 abcde	6191 ± 638.2 b
11		b50	2417 ± 436.9 ab	4142 ± 863.6 cd	5448 ± 596.2 abcde	6462 ± 638.2 ab
12		b100	1416 ± 504.5 ab	5869 ± 747.9 abcd	6024 ± 596.2 abcde	7812 ± 638.2 ab
13		CuCl2 50	2900 ± 436.9 a	6252 ± 747.9 abcd	4048 ± 596.2 cde	7184 ± 638.2 ab
14		CuCl2 100	2267 ± 436.9 ab	5950 ± 747.9 abcd	4057 ± 596.2 cde	6367 ± 638.2 ab
15	100	0	657 ± 436.9 ab	5506 ± 747.9 bcd	5101 ± 596.2 abcde	7381 ± 638.2 ab
16		n50	800 ± 504.5 ab	6564 ± 747.9 abcd	5293 ± 596.2 abcde	6539 ± 638.2 ab
17		n100	1586 ± 436.9 ab	5202 ± 863.6 bcd	3337 ± 596.2 e	7412 ± 638.2 ab
18		b50	1758 ± 436.9 ab	4891 ± 747.9 bcd	4275 ± 596.2 bcde	8444 ± 638.2 ab
19		b100	441 ± 504.5 b	6840 ± 747.9 abc	4937 ± 596.2 bcde	9780 ± 638.2 a
20		CuCl2 50	1528 ± 436.9 ab	6077 ± 747.9 abcd	4601 ± 596.2 abcde	8643 ± 638.2 ab
21		CuCl2 100	1057 ± 504.5 ab	3993 ± 747.9 cd	3919 ± 596.2 cde	6328 ± 737.0 b

± represent standard error.

Table S 15

Aluminum concentrations [Al] in pods, leaves, stems and roots of green pea plants exposed until maturity to 1) control 1, 2)*n*CuO 50 mg/kg, 3) *n*CuO 100 mg/kg, 4)*b*CuO 50 mg/kg, 5)*b*CuO 100 mg/kg, 6)CuCl□ 50 mg/kg, 7)CuCl□ 100 mg/kg, 8)10 µM IAA (control 2) , 9) 10 µM IAA+ *n*CuO 50 mg/kg, 10) 10 µM IAA + *n*CuO 100 mg/kg, 11)10 µM IAA + *b*CuO 50 mg/kg, 12) 10 µM IAA + *b*CuO 100 mg/kg, 13) 10 µM IAA + CuCl□ 50 mg/kg, 14) 10 µM IAA + CuCl□ 100 mg/kg, 15) 100 µM IAA (control 3), 16) 100 µM IAA + *n*CuO 50 mg/kg, 17) 100 µM IAA + *n*CuO 100 mg/kg, 18)100 µM IAA + *b*CuO 50 mg/kg, 19) 100 µM IAA + *b*CuO 100 mg/kg, 20) 100 µM IAA + CuCl□ 50 mg/kg, and 21) 100 µM IAA + CuCl□ 100 mg/kg. Results are means ± SE. Letters indicate statistical differences and * indicate statistical differences compare to their respective control ($p \leq 0.05$).

Treatments	[Al] mg/kg in different plant tissues

ID	IAA (μM)	Cu Compound (mg/kg d wt)	Pods	Leaves	Stems	Roots
1	0	0	5.5 ± 1.9 a	57.9 ± 11.4 ab	33.1 ± 8.2 b	1203.8 ± 176 a
2		n50	5.1 ± 1.9 a	62.5 ± 11.4 ab	33.3 ± 7.1 b	1395.5 ± 176 a
3		n100	6.1 ± 1.9 a	62.2 ± 11.4 ab	42.1 ± 7.1 ab	1379.8 ± 176 a
4		b50	5.1 ± 1.9 a	63.3 ± 11.4 ab	51.2 ± 7.1 ab	1267.0 ± 176 a
5		b100	3.8 ± 1.9 a	60.6 ± 11.4 ab	47.8 ± 7.1 ab	1600.7 ± 176 a
6		CuCl2 50	4.8 ± 1.9 a	36.7 ± 11.4 b	49.4 ± 7.1 ab	1203.4 ± 176 a
7		CuCl2 100	4.5 ± 1.9 a	56.6 ± 11.4 ab	52.3 ± 7.1 ab	1075.9 ± 176 a
8	10	0	5.9 ± 1.9 a	62.8 ± 11.4 ab	62.9 ± 7.1 ab	1217.8 ± 176 a
9		n50	5.4 ± 2.2 a	45.2 ± 11.4 ab	53.3 ± 7.1 ab	1274.9 ± 176 a
10		n100	6.2 ± 1.9 a	50.3 ± 11.4 ab	47.1 ± 7.1 ab	873.7 ± 176 a
11		b50	11.3 ± 1.9 a	45.7 ± 11.4 ab	57.8 ± 7.1 ab	1054.2 ± 176 a
12		b100	8.2 ± 2.2 a	49.9 ± 11.4 ab	42.9 ± 7.1 ab	1417.2 ± 176 a
13		CuCl2 50	5.2 ± 1.9 a	33.1 ± 11.4 b	51.9 ± 7.1 ab	1202.0 ± 176 a
14		CuCl2 100	4.8 ± 1.9 a	100.4 ± 11.4 a	41.8 ± 7.1 ab	1221.1 ± 176 a
15	100	0	3.7 ± 1.9 a	43.6 ± 11.4 ab	60.0 ± 7.1 ab	1015.2 ± 176 a
16		n50	13.3 ± 2.2 a	51.2 ± 11.4 ab	74.1 ± 7.1 a	997.0 ± 176 a
17		n100	4.9 ± 1.9 a	49.5 ± 11.4 ab	37.2 ± 7.1 ab	1161.8 ± 176 a
18		b50	5.9 ± 1.9 a	39.4 ± 11.4 b	55.7 ± 7.1 ab	1194.4 ± 176 a
19		b100	4.9 ± 1.9 a	56.1 ± 11.4 ab	39.9 ± 7.1 ab	1490.9 ± 176 a
20		CuCl2 50	4.8 ± 1.9 a	32.0 ± 11.4 b	52.9 ± 7.1 ab	1200.8 ± 176 a
21		CuCl2 100	4.8 ± 2.2 a	30.0 ± 13.2 b	33.6 ± 7.1 b	1066.3 ± 176 a

Table S 16 Effects on seed germination From plants that were grown in soil amended in Cu compound (nCuO, bCuO, and $CuCl_2$) at different concentrations (50 and 100 mg/kg of soil) and enhanced with IAA (0, 10, and 100 μM). Results are means ± SE. Letters indicate statistical differences ($p \leq 0.05$).

ID	IAA (μM)	Cu Compound (mg/kg d wt)	germination
1	0	0	3.8 ± 0.74 a
2		n50	5.5 ± 0.74 a
3		n100	4.3 ± 0.74 a
4		b50	4.0 ± 0.74 a
5		b100	4.5 ± 0.74 a
6		CuCl2 50	3.8 ± 0.74 a
7		CuCl2 100	5.5 ± 0.74 a
8	10	0	5.8 ± 0.74 a
9		n50	5.3 ± 0.74 a
10		n100	6.5 ± 0.74 a
11		b50	5.5 ± 0.74 a
12		b100	4.5 ± 0.74 a
13		CuCl2 50	4.8 ± 0.74 a
14		CuCl2 100	6.0 ± 0.74 a
15	100	0	5.3 ± 0.74 a
16		n50	4.3 ± 0.74 a
17		n100	5.5 ± 0.74 a
18		b50	4.8 ± 0.74 a
19		b100	4.5 ± 0.74 a
20		CuCl2 50	3.8 ± 0.74 a
21		CuCl2 100	4.3 ± 0.74 a

± represent standard error.

Vita

Born in El Paso, TX., and raised half of her life in Cd. Juarez Chihuahua, Mexico. Loren Ochoa graduated High School from Bel Air High School. After graduating, she entered to El Paso Community College (EPCC) and graduated with her Associates degree. Most recently she graduated from the University of Texas at El Paso (UTEP), and obtained her Bachelor's degree in Biology on 2013 with a GPA of 3.43/4. She volunteered in a herpetology and entomology laboratories for a semester. Right after that, she started her Master's degree in Environmental Science under the mentorship of Dr. Jorge Gardea-Torresdey. Her research focus on the effects of indole-3-acetic acid (a phytohormone) and copper oxide nanoparticles (nCuO) in the response of green pea (*Pisum sativum* L.) plants and seeds. This study aims for the better understanding of the effects that could be cause at the physiological and biochemical level in green pea. Out of these study, two manuscripts were able to be produced in order to provide the information gathered to the scientific field (in process).

1. Ochoa, L. Medina-Velo, I.A., Peralta-Videa, J.R., Gardea-Torresdey, J.R. (2017). Evaluating the interaction of phytohormone índole-3-acetic acid in the response of soil grown green pea (*Pisum sativum* L.) plants to CuO nanoparticles exposure.

2. .Ochoa, L. Medina-Velo, I.A., Peralta-Videa, J.R., Gardea-Torresdey, J.R. (2017). Nutritional values in green pea (*Pisum sativum* L.) seeds from plant exposed to CuO nanoparticles and indole-3-acetic acid.

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This thesis/dissertation was typed by Loren Ochoa.