




Comparison of foliar spray and soil irrigation of biogenic CuO nanoparticles (NPs) on elemental uptake and accumulation in lettuce

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Abstract

Nanoparticles (NPs) can be used in several ways in agriculture, including increasing production rates and improving nutritional values in plants. The present study aims to clarify how biogenic copper oxide nanoparticles (CuO NPs) applied by two routes of exposure (foliar spray and soil irrigation) affect the elemental uptake by lettuce. In vivo experiments using lettuce ($n = 4$) were performed with CuO NPs in comparison with copper salt (CuSO_4), considering a final mass added of 20 mg of CuO per plant. The elemental composition of roots was mostly affected by the soil irrigation exposure for both Cu forms (NPs and salt). Neither Cu form added by soil irrigation was translocated to leaves. Copper concentration in leaves was mainly affected by foliar spray exposure for both Cu forms (NPs and salt). All Cu forms through foliar spray were sequestered in the leaves and no translocation to roots was observed. Foliar spray of CuO NPs caused no visual damage in leaves, resulted in less disturbance of elemental composition, and improved dry weight, number of leaves, CO_2 assimilation, and the levels of K, Na, S, Ag, Cd, Cr, Cu, and Zn in leaves without causing significant changes in daily intake of most elements, except for Cu. Although Cu concentration increased in leaves by foliar spray of CuO NPs, it remained safe for consumption.

Keywords Biogenic nanoparticles · Multielemental determination · Fertilization · Food safety · Food security · Copper oxide nanoparticles

Introduction

In recent decades, nanotechnology has received considerable attention as a feasible tool in many areas of knowledge,

including agriculture (Gajjar et al. 2009; Wang et al. 2016). Previous studies indicated the potential use of metallic and mining reject nanoparticles (NPs) to increase agricultural production rates (Dalmora et al. 2016a, b; Ferrari et al. 2019; Gredilla et al. 2019; Plata et al. 2021), the efficiency of natural resources use, reduction in waste generation, development of fertilizers, nanopesticides, nanostructured materials for the treatment of agricultural waste and nanosensors (Adisa et al. 2019; Arruda et al. 2015; Kah et al. 2019; Khot et al. 2012; Rodrigues et al. 2017; White and Gardea-Torresdey 2018).

Copper-based NPs in particular have drawn considerable attention for agriculture purposes due to their antimicrobial properties (Hong et al. 2015; Servin et al. 2017). Moreover, Cu is one of the essential trace elements that play a significant role in the regulation of many physiological and biochemical processes in plants (Kaewchangwat et al. 2017; Singh et al. 2013). However, Cu deficiency can harm plant metabolism, resulting in low crop yield and physiological disturbance (Kaewchangwat et al. 2017; Singh et al. 2013). In addition, most Cu compounds are highly toxic (Zamberlan et al. 2020).

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Inorganic NPs have been investigated as alternatives to alleviate the undesirable effects of the accumulation of some potentially toxic elements (PTEs); for example, Si alleviated the effects of Cr in peas (Tripathi et al. 2015), As in maize (Tripathi et al. 2016), Cd in rice (Rizwan et al. 2019), and Pb and Cd in sunflower (Mousavi et al. 2018). In addition, TiO₂ NPs alleviated the effects of Cd in soybean (Singh and Lee 2016), Pb in rice (Cai et al. 2017), and Cd in rice (Rizwan et al. 2019). The application of NPs in the presence of these PTEs may also result in the reduction of some elements essential to plant nutrition such as Ca, N, P, Zn, Mn, Fe, S, K, and Na (Hayes et al. 2020; Hu et al. 2020). Therefore, studies have presented a great diversity of negative and positive effects after the application of inorganic synthetic and natural NPs (Abollino et al. 2007; Cortés et al. 2020; Oliveira et al. 2019; Ramos et al. 2020).

It is important to understand the application effects of NPs on the absorption of essential elements and PTEs in lettuce due to the high consumption of this vegetable worldwide (Hong et al. 2015). Lettuce is well known to accumulate PTEs, such as Cd and Pb (Zhang et al. 2013). The effects of commercial copper oxide (CuO) NPs on the elemental composition of lettuce have been investigated (Hong et al. 2015; Zhao et al. 2016a). The exposure of hydroponic lettuce to CuO NPs (20 mg L⁻¹) resulted in higher absorption of Cu, reduced P and Fe accumulation in shoots, and increased S in the roots (Hong et al. 2015). Zhao et al. (2016a) exposed the lettuce leaves grown in soil to commercial Cu(OH)₂ nanopesticides for 1 month and reported a significant accumulation of K and Zn in vascular tissues and a decrease in Mg.

In this study, we evaluated the effects of CuO NPs synthesized by the green chemical route on lettuce. This biologically friendly route involved the use of green tea extract as a reducing and coating agent. Green tea extract has antioxidant phytochemicals such as catechin, which allow CuO NP formation (Rolim et al. 2019a). In addition, antioxidant molecules derived from green tea also act as coating agents to avoid NP aggregation, thereby increasing their biocompatibility. Studies into the effects of the exposure of lettuce to biogenic CuO NPs are still scarce. Considering this scenario, the present study aimed to clarify how biogenic CuO NPs applied by different routes of exposure (foliar spray and soil irrigation) interact with different elements. The objectives were (1) to synthesize and characterize biogenic CuO NPs, (2) to evaluate the effect of CuO NPs and CuSO₄ on productivity and elemental concentrations on lettuce grown in pots under greenhouse conditions, and (3) to estimate the daily nutrient intake in lettuce tissues. To the best of our knowledge, this is the first report to compare the foliar spray and soil irrigation administration of green tea synthesized CuO NPs compared to CuSO₄ on lettuce growth and metal uptake.

Materials and methods

Reagents

Copper chloride II (CuCl₂) used for the synthesis of NPs and copper sulfate (CuSO₄) used for plant application were purchased from Sigma–Aldrich (St. Louis, MO, USA). Green tea powder (*Camellia sinensis*) used for green synthesis was obtained from Sumioka Shokuhin Kabushikikaisha (Hiraguti, Japan). Sodium hydroxide (NaOH) was obtained from Synth (Diadema, SP, Brazil). Nitric acid (HNO₃) (Synth, São Paulo, Brazil) was previously purified with the SavillexTM DST-1000 sub-boiling distillation system (Minnetonka, USA). High purity deionized water (resistivity 18.2 MΩ cm⁻¹) used throughout the experiments was obtained by the Elga water purification system (ELGA, Ubstadt-Weiher, Germany).

Green synthesis of CuO nanoparticles

An aqueous solution of CuCl₂ (93.0 mmol L⁻¹) was added dropwise into a suspension of green tea extract (2.5 mg mL⁻¹). The volumetric proportion of CuCl₂ and green tea extract was 1 to 2, respectively. The pH of the final suspension was adjusted to 5.5 using NaOH (1 mol L⁻¹), then the mixture was stirred for 15 min by magnetic stirring. The final mixture was centrifuged. The black proportion of precipitate of CuO nanoparticles (CuO NPs) was freeze-dried (Pelegriño et al. 2020).

Characterization of CuO NPs

The hydrodynamic size, polydispersity index (PDI), and zeta potential values of CuO NPs were analyzed by dynamic light scattering (DLS, Zetasizer Nano ZS, Malvern Instruments Co, UK) (Pelegriño et al. 2020; Rolim et al. 2019a, b). The morphology of CuO NPs was obtained by transmission electron microscopy (TEM) at 20 kV (JEM-2100 TEM, Jeol Ltd.) and analyzed using the ImageJ software (Pelegriño et al. 2020; Rolim et al. 2019a). Copper concentration of the biosynthesized CuO NPs was determined by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7900, Hachioji, Japan). The extraction method and Cu analysis were performed according to Souza et al. (2019). Briefly, triplicate samples of approximately 15 mg of CuO NP powder each were placed in 50-mL Teflon tubes. Then, 4 mL of HNO₃ (65% v v⁻¹) were added and the tubes were closed. The extractions were carried out for 2 h at room temperature (25 °C). The volume was made up to 50 mL with type 1 water. Finally, the solution was diluted 1000 times and Cu concentration was determined by ICP-MS. This procedure was performed in triplicate. Similar analytical procedures have been reported by several previous works (Duarte et al. 2019; Oliveira et al. 2018; Gasparotto et al. 2018; Nordin et al. 2018; Wilcox et al. 2015).

Soil characterization

The soil samples ($n = 3$) were collected from an urban agriculture field in the vicinity of a petrochemical plant in Santo André, SP, Brazil (23° 64' S; 46° 49' W). This site has been used as an urban garden for the last three decades, and the soil is under the influence of several metals released into the environment by the petrochemical industry and vehicular traffic. To simulate a field condition, the soil was prepared just exactly as the farmers on the site recommended as follows: a bed was separated to provide enough soil amounts for characterization and pot experiments. It was prepared by mixing the local soil and organic amended compounds (compost and spent mushroom substrate) (1:1) and sieving (2 mm).

For soil characterization, approximately 1000 kg was collected. Then, the soil was oven-dried at 40 °C until constant mass, quartered, homogenized, and sieved with a stainless mesh (2 mm). Soil texture, pH, organic matter (OM), cation exchange capacity at pH 7 (CEC), sum of bases (SB), base saturation (V %), and potential acidity (H + Al) were determined according to Embrapa (Donagema et al. 2011; van Raij et al. 2001).

In addition, several chemical elements were quantified in soil (Ag, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mo, Na, Ni, P, Pb, S, Sb, Se, U, V, and Zn) sieved with a stainless mesh (150 mm). For this purpose, the United States Environmental Protection Agency (USEPA) 3051A procedure (US-EPA Method 3051A 2007) was performed, with some modifications according to Segura et al. (2016) and Suda and Makino (2016). About 500 mg of soil was pre-digested for 48 h with 10 mL of concentrated HNO₃. Then, the samples were heated in a digesting block at 175 °C for 15 min. The digested samples were diluted to 50 mL with type 1 water.

The elements were determined using an inductively coupled mass spectrometer (Agilent 7900, Hachioji, Japan). A stock solution containing all elements (10 mg L⁻¹) (Perkin Elmer, USA) was used to prepare the calibration curve according to Paniz et al. (2018). To ensure the reliability of the results (accuracy and precision), the certified reference materials TILL-4 (soil) (Canadian Certified Reference Materials, Vancouver, Canada) were analyzed. The results of the analysis of the CRMs were statistically consistent with the certified values.

The mineralogical composition identification of the soil was determined by the powder method using an X-ray diffractometer (PANalytical, model: Xpert Pro, The Netherlands) with an X'Celerator detector using Cu-K α radiation ($\lambda = 1,542 \text{ \AA}$, 2,2 kW). Crystalline phase identification was done by comparison with the ICDD (International Center for Diffraction Data) and PANalytical inorganic crystal structure databases.

In vivo bioassays

Lettuce seedlings (*Lactuca sativa* L. (cv. Vanda)) were grown under greenhouse conditions controlling for temperature and humidity (25 °C and 50–60%, respectively). Seedlings were grown for 30 days and transplanted to individual pots of 3.4 dm³. Plants were watered daily at ~60% of their field capacity. We focus on the comparison of CuO NPs and CuSO₄ salt applied in two different exposure routes: (i) spray on leaves (foliar application) and (ii) soil irrigation water directly added to soil by using an automatic pipette (soil irrigation). The treatments were CuO NPs by (i) foliar spray application and (ii) soil irrigation; CuSO₄ by (iii) foliar spray application and (iv) soil irrigation; and (v) control without Cu exposure. The 5 groups were divided to contain 4 replicates randomly distributed in blocks composed of 3 rows of 10 m spaced 0.30 m apart.

Particles were added to diluent (water), and suspensions were sonicated for 10 min at 2800 rpm. The treatments were applied after 2 weeks of acclimatization; thus, the exposure started at day 45. The approximate volume amount for each pot was 15 mL per event. The exposure frequency was twice per week. A total of eight sprays were done by harvest time and the final concentration added by the treatment was 20 mg of CuO per plant and the equivalent mass of Cu was calculated to define the mass of CuSO₄ added per plant. This concentration was defined based on previous studies by our research group (Pelegriño et al. 2020). At day 75, the plants were harvested.

Growth parameters

Fresh and dry weight, longitudinal and radial length, and number of leaves

After the in vivo bioassays, the lettuce samples were measured using a metric scale as to the longitudinal and radial length (cm), fresh and dry weight (FW, DW) (g) (excluding senescent leaves, if any) and the number of leaves was counted manually during the harvest stage.

Leaf gas exchange

Leaf gas exchange was measured at the end of the experiment, 2 days after the 8th application. For this, we used an infrared gas analyzer (Li-6400, Licor, Lincoln, NE, USA) attached to a modulated fluorometer (6400-40 LCF, Licor, Lincoln, NE, USA). Leaf CO₂ assimilation (A), stomatal conductance (gs), intercellular CO₂ concentration (Ci), and transpiration (E) were measured under PPFD of 500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ and an air CO₂ concentration of 400 $\mu\text{mol mol}^{-1}$. The plants were well hydrated before the measurements. The instantaneous carboxylation efficiency (k) was given by the rate between

leaf CO_2 assimilation and intracellular CO_2 partial pressure (A/ Ci). The measurements were performed between 8:30 and 11:00 a.m. The vapor pressure difference between leaf and air (VPDL) was 2.2 ± 0.3 kPa and leaf temperature was 29.0 ± 1.0 °C during the evaluations.

Elemental determination in lettuce and soil

At the end of the experiment, the heads of lettuce were separated from the roots. Then, the root samples were separated from the soil. The plant samples (leaves and roots) were washed under running water and the excess water was manually removed. In the laboratory, plant samples and soil were oven-dried at 40 °C until constant mass. Finally, soil samples were quartered, homogenized, and sieved with a stainless mesh (150 mm).

After drying, plant tissues were prepared and analyzed according to Paniz et al. (2018). The roots and leaves were cut into fine pieces. About 200 mg of sample was pre-digested for 48 h in 2 mL of concentrated HNO_3 previously purified in a sub-boiling distillation system. The samples were heated in a graphite digester block for 4 h at 95 °C. After cooling at room temperature, the samples were diluted in 40 mL with type 1 water. Soil samples were prepared and analyzed as described in detail in Section 2.4.

The elements were determined using an inductively coupled mass spectrometer (Agilent 7900, Hachioji, Japan). A stock solution containing all elements (10 mg L^{-1}) (Perkin Elmer, USA) was used to prepare the calibration curve according to Paniz et al. (2018). To ensure the reliability of the results (accuracy and precision), the certified reference materials (CRMs) NIST 1573 (tomato leaves) (National Institute of Standards and Technology, Gaithersburg, MD, USA) were analyzed. These materials were subjected to the same methods used for the analysis of plant tissues. Moreover, blank samples were assayed throughout all experiments to check for possible contamination problems. The results of the analysis of the CRMs were statistically consistent with the certified values.

Estimated daily intake of chemical elements from lettuce

The estimated daily intake of some elements (those that presented significant differences among pot groups) through lettuce consumption was calculated by Eq. 1 (Xu et al. 2013).

$$\text{EDI} = \text{DI}_{\text{vegetables intake}} \times C_{\text{element}} \quad (1)$$

where EDI is the estimated daily intake for each element, expressed as mg day^{-1} . DI represents the average daily intake of lettuce, which was assumed to be 40 g day^{-1} FW (approximately four leaves). This value is in good agreement with the

daily lettuce consumption in the USA of 41 g day^{-1} FW (Zhao et al. 2016a). Finally, C_{element} is the average element concentration in lettuce leaves ($n = 4$) in mg g^{-1} FW.

Statistical analysis

The dataset consisted of 72 sample cases (4 replicates \times 3 treatments \times 2 exposure routes \times 3 matrices) by 24 variables (elemental content) distributed by treatment (control, CuO NPs, and CuSO_4), exposure (foliar and soil irrigation), and matrix (root and leaf). A two-way analysis of variance (ANOVA) was applied by sample matrices (M) where metals were measured to test if statistical differences were observed by the considered factor: treatment (T), exposure route (E), and their interaction ($E \times T$). In all the tests, a significance level of 0.05 was considered.

Results and discussion

Characterization of CuO NPs

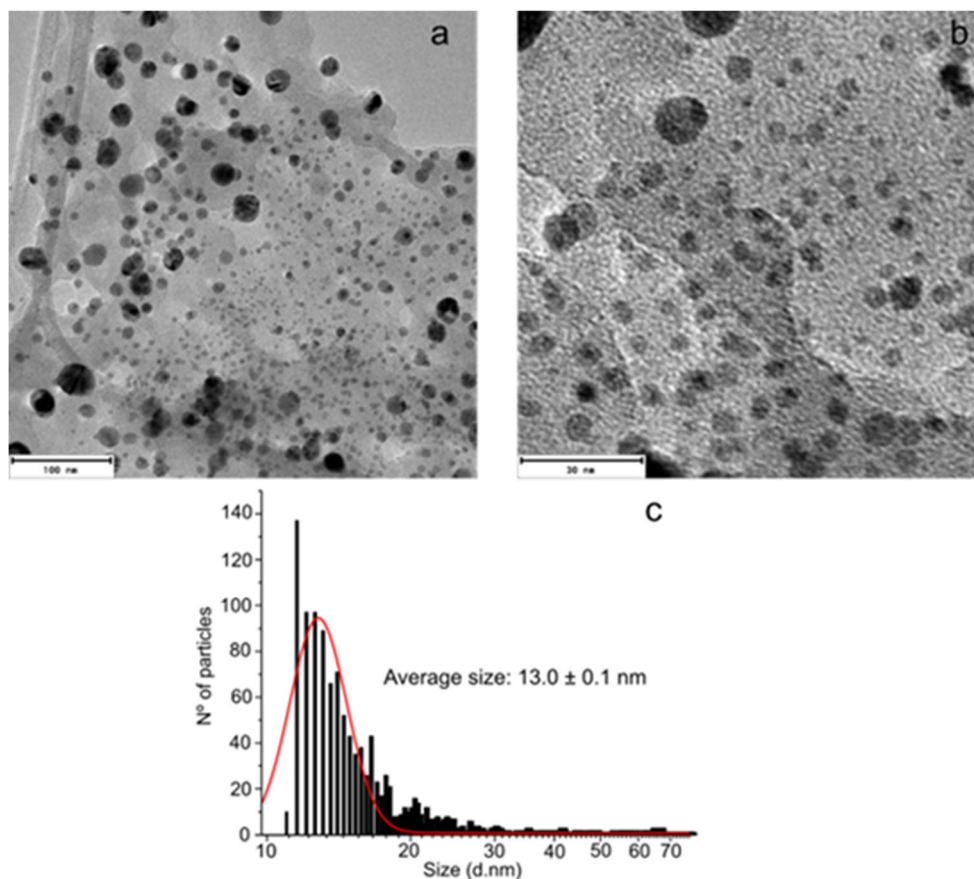
The hydrodynamic size of CuO NPs was 74.6 ± 1.5 nm with a PDI of 0.28 ± 0.06 and a zeta potential of -20.8 ± 0.5 mV. These values are similar to those reported for commercial CuO NPs (Hong et al. 2015; Singh and Kumar 2016). The total copper content in CuO NPs (mass ratio) obtained by ICP-MS was $52 \pm 5\%$. Figure 1a and b shows representative images of CuO NPs using TEM. The NPs had a spherical shape and were well dispersed (Fig. 1a, b) with an average size of 13.0 ± 0.1 nm (Fig. 1c). Other studies using green synthesis also showed a similar NP shape, for example, Dey et al. (2019) showed spherical shape CuO NPs synthesized using *Azadirachta indica* leaves, and Naika et al. (2015) showed similar results for CuO NPs synthesized using *Gloriosa superba* L. extract. These results are similar to our previously published paper (Pelegrino et al. 2020).

Thus, the green synthesis of CuO NPs was successfully performed and yielded NPs with good stability. Moreover, it was possible to obtain NPs with a size and shape similar to commercial NPs with the advantage of using a green synthesis and increasing biocompatibility due to the green tea coating.

Soil characterization

The geochemical composition of the soil used in the pot experiment is reported on the supplementary material (Table S1). In general, most samples consist of moderately to slightly acidic soils, with a pH of 5.9 on average. The results showed about 338 g kg^{-1} of clay, high sand content 477 g kg^{-1} on average and, in general, a median silt content with an average of 186 g kg^{-1} . The soil texture was classified as sandy-clay-loam soil, which is considered a soil of moderately fine

Fig. 1 Representative images of CuO NPs (a) with a 100-nm scale and (b) with a 30-nm scale. c Represents a size distribution adjusted to the log-normal curve



texture (Dos Santos et al. 2018), and PTE retainability is generally higher in fine-textured soils than coarse-textured soils due to the presence of more pore spaces (McBride et al. 2014).

The OM content was 58 g kg^{-1} , which is considered high for tropical soils (Fadigas et al. 2002) facilitating PTE and NP attachment. The CEC, which expresses the soil's capacity to retain cations, showed an average of $185 \text{ mmol}_c \text{ dm}^{-3}$; this value is also considered high for tropical soils according to Fadigas et al. (2002). These authors studied 162 Brazilian clayey soils and observed that 79% of the samples had a CEC lower than $100 \text{ mmol}_c \text{ dm}^{-3}$. The high CEC value of the soil used in our study may be attributed to the high OM content and to the presence of clay minerals.

The X-ray diffraction results (Fig. S1) of soil showed the presence of higher intensity peaks of quartz (SiO_2) and gibbsite ($\text{Al}(\text{OH})_3$). Clay minerals such as kaolinite ($\text{Al}_2(\text{Si}_2\text{O}_5)(\text{OH})_4$), vermiculite ($\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$), and muscovite ($\text{KAl}_2(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH})_2$) were also identified. A lower abundance of anatase (TiO_2) was observed.

Primary minerals such as kaolinite clays tend to have moderate CEC ($10\text{--}150 \text{ mmol}_c \text{ dm}^{-3}$), while vermiculite has a 2:1 crystalline structure with high CEC ($1000\text{--}2000 \text{ mmol}_c \text{ dm}^{-3}$) (Brady et al. 2008), which is a consequence of surface and interlayer ion exchange processes as well as isomorphic

substitutions. Such properties, associated with high surface areas, have made these materials capable of attaching PTEs depending on soil acidity and may be also a target of attachment of metallic NPs (Geitner et al. 2020).

The base saturation was high, 82%, and the soil can be classified according to the current Brazilian soil classification system as eutrophic soil ($V \geq 50\%$) (Dos Santos et al. 2018). The concentration of exchangeable phosphorus in the urban garden soil samples was 274 mg dm^{-3} , which is considered a sufficient value for productive cultivation (Van Raij 2011). Overall, this soil presented characteristics to supply nutrients to cultivation, since the farmers from this urban agriculture field, where the soil was collected, periodically use acidity correctives, mineral, and organic fertilizers. However, this can facilitate the incursion of contaminants such as PTEs.

Total contents of PTEs in the soil rank in the following order: $\text{Ba} > \text{Zn} > \text{Cu} > \text{V} > \text{Pb} > \text{Cr} > \text{Ni} > \text{As} > \text{Co} > \text{Mo} > \text{Se} > \text{Sb} > \text{Cd} > \text{Ag}$. There is no international consensus regarding soil PTE guidelines. The barium level (662 mg kg^{-1}) is above the intervention value for agricultural purposes (500 mg kg^{-1}) described by the local environmental protection agency (Environmental Protection Agency of the State of São Paulo, Brazil) (CETESB 2016). This represents the amount of an element in the soil or groundwater that may pose direct or

indirect potential risks to human health. Copper, Se, Pb, and Zn levels are above the São Paulo State Prevention Values (SP-PV), but are still considered safe for agricultural activities (CETESB 2016). High levels of Ba were previously reported in the Brazilian urban soil studies (Figueiredo et al. 2011; Lange et al. 2017; Lange et al. 2018), since this element is applied in manufactured materials such as tiles, glass, bricks, pigments, paints, brake lines, and auto parts.

Productivity of lettuce

Fresh and dry weight, longitudinal and radial length, and number of leaves

The productivity parameters of lettuce plants were evaluated by FW and DW, longitudinal and radial length, and the number of leaves (Fig. 2a–e). A two-way ANOVA (data not presented) showed that the effect of treatment was prevalent for DW (CuO NPs significantly improved DW among pot groups). Conversely, the number of leaves was affected mostly by the route of exposure, where foliar spray improved the number of leaves among pot groups. A significant increase in DW (46%) and in the number of leaves (18%) was observed

in the group exposed to CuO NPs by foliar spray compared to the control (Fig. 2a, d).

Neither CuO NP nor CuSO₄ application by soil irrigation caused any significant changes in productivity parameters (Fig. 2). Foliar spray led to a significant increase (8 ± 1%) in fresh weight, regardless of the form of Cu (Nano or salt). For longitudinal length, there was a statistically significant difference among the treatments ($p < 0.05$): CuSO₄-treated pots significantly improved longitudinal length among pot groups. Neither treatment, exposure, nor their combined effect affected the radial length. Figure S2 shows representative images of different treatments at the final application (8th application).

Previous studies reported positive and negative impacts of NPs on productivity parameters. The application of CuO NPs enhanced root length and biomass of lettuce (Hong et al. 2015; Wang et al. 2019). However, several studies have described commercial CuO NPs application as reducing root and longitudinal length, biomass, and water content in lettuce (Dimkpa et al. 2013; Hong et al. 2015; Trujillo-Reyes et al. 2014). Hafeez et al. (2015) evaluated the use of biogenic Cu NPs (20 nm) in wheat growth. The concentration of 30 mg kg⁻¹ doubled the leaf area and the chlorophyll content. On the other hand, concentrations of Cu NPs above 30 mg kg⁻¹ can result in

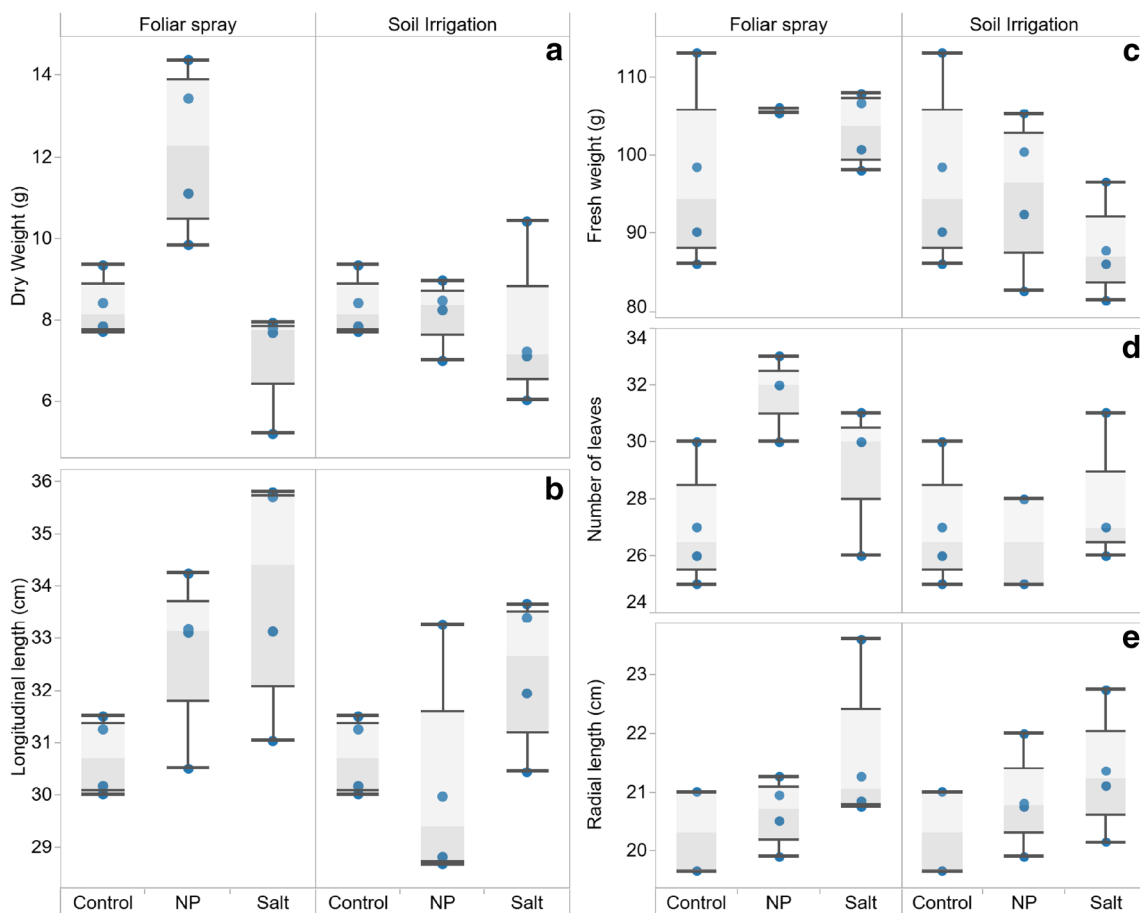


Fig. 2 Productivity parameters of lettuce plants treated with CuO NPs in comparison with copper salt and the control ($n = 4$)

phytotoxic effects on the plants (Hafeez et al. 2015). In comparison with the results obtained by other inorganic NPs, the use of TiO₂ NPs in spinach doubled the FW and DW compared to the control (Yang et al. 2007). Furthermore, the application of four rare earth oxide NPs (CeO₂, La₂O₃, Gd₂O₃, and Yb₂O₃) implied in a reduction of 75% to 90% of length in lettuce plants (Ma et al. 2010). Our findings suggested that foliar spray of the biogenic CuO NPs has beneficial impacts on lettuce productivity.

Leaf gas exchange

The leaf gas exchange markers studied were CO₂ assimilation, transpiration, and time-course of stomatal conductance (Fig. 3). Figure 3a shows that CO₂ assimilation slightly increased after NP exposure via foliar application, and as previously discussed, this pot group also increased the DW and number of leaves (Fig. 2a, d). Therefore, this treatment/exposure did not present toxicity to lettuce; actually, it showed a positive effect on productivity and CO₂ assimilation. However, CO₂ assimilation was not affected for CuSO₄-treated pots exposed by foliar spray. Via soil irrigation, the NPs did not change the CO₂ assimilation; however, the application of CuSO₄

significantly decreased it in comparison with control. Rawat et al. (2018) showed similar results after treating bell pepper. They also reported a decrease in CO₂ assimilation after salt application and no disturbance of it with Cu NPs. The CO₂ assimilation was 34% higher using foliar application than soil irrigation (Fig. 3a).

Small changes without statistical significance were observed for transpiration (Fig. 3b). For this parameter, there was a high variability between measurements, which may be related to changes in environmental conditions when each plant was measured. The stomatal conductance slightly increased after NP and salt treatments via foliar and soil irrigation exposure. In addition, it was 28% higher using the foliar than soil irrigation application (Fig. 3c). NPs applied via foliar spray could cause entrapment of NPs in the cuticle or encompass penetration through stomata (Priyanka et al. 2019). This did not occur in our study, since the results showed a non-significant difference between stomatal conductance treated with NPs and salt via foliar or soil irrigation in comparison with control (Fig. 3c). Overall, all treatments showed a nontoxic effect for lettuce regarding its productivity and gas exchange parameters.

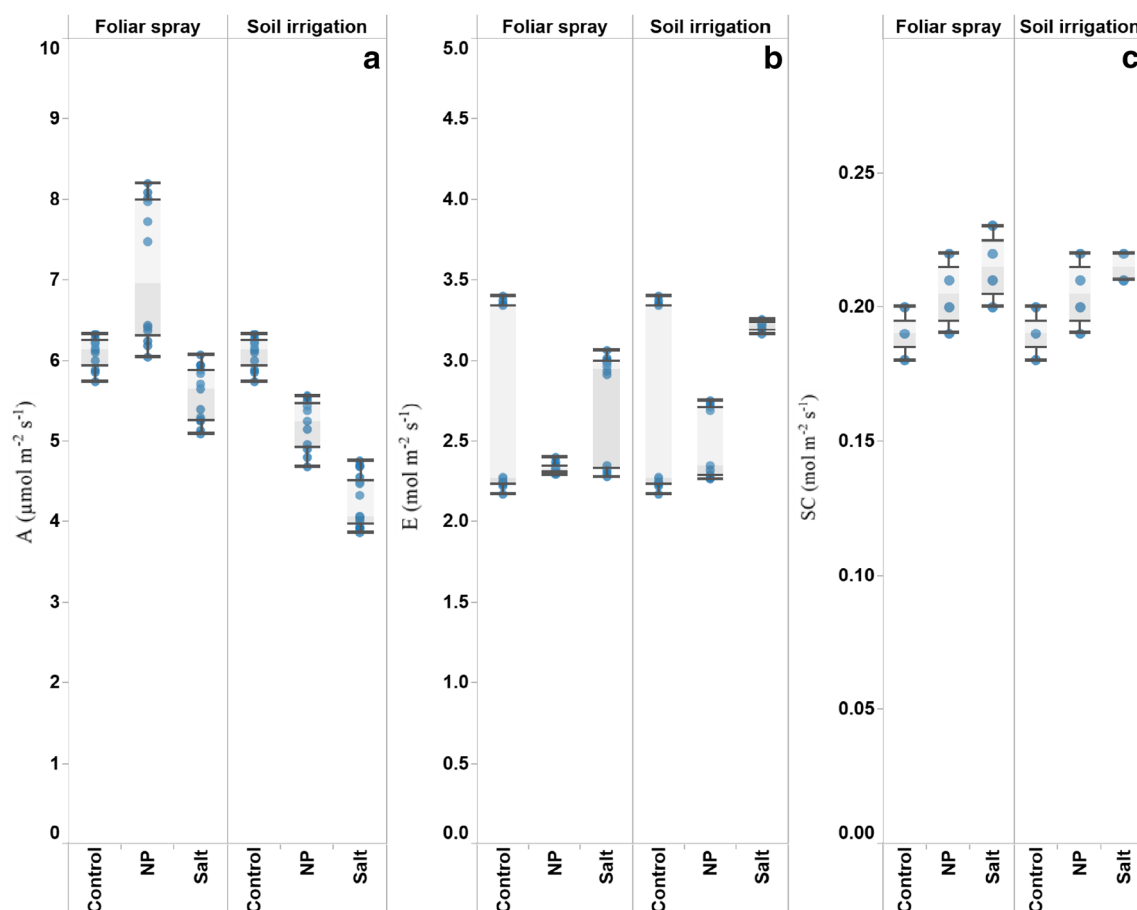


Fig. 3 a Leaf CO₂ assimilation (A), b transpiration (E), and c time-course of stomatal conductance (gs) of lettuce plants in the control, treated with CuO NPs or CuSO₄ via foliar application or soil irrigation ($n = 4$)

Determinations of macro and trace elements in samples

Regarding the elemental average concentrations in leaves and roots, the detailed information is reported in Table 1. As seen in this table, elemental content is influenced by treatments and exposure in different ways compared to the control. Soil elemental composition was also determined after harvest (Table S2). The results showed that Cu concentration in soil was slightly lower in all treatment samples (ranging from 89 to 101 mg kg⁻¹) than the control (102 to 104 mg kg⁻¹); thus, Cu-treated pots did not cause a significant increase of Cu in soil.

Exposure and treatment effects on elemental variability

The elemental concentration variability by the exposure route (E), by the treatment (T), and their combined effect (E × T) was assessed by a two-way ANOVA (Tables S3 and S4). Figure 4 summarizes the significant changes in the elemental concentration of root and leaf tissues compared to the corresponding controls, as proposed previously in the study by Cota-Ruiz et al. (2020). The results showed that (T), (E), and the interactions between them all had significant effects on the leaf and root mineral profile.

Neither treatment, exposure, nor their combined effect influenced As, Ba, Cd, Co, Cr, Fe, Mn, Na, Ni, Li, Pb, Sn, or U concentrations in roots (Table 1). Copper, P, Sb, and V concentrations in roots were significantly affected only by (E), mainly by soil irrigation, except for Sb, the foliar application of which displayed a more significant effect than soil irrigation. The effect of (T) (CuO NPs) was only significant on K concentrations in roots. Treatments (NPs and salt) exhibited a more significant effect than (E) for Mo, S, and Zn. Calcium content in roots was significantly affected by (E), (T) and their interaction (E × T); however, (T) (both NPs and salt) had a more significant effect than the other two factors. Magnesium and Ag contents in roots were significantly affected by (E) and (E × T); however, for Ag, the (E) (soil irrigation) showed a more significant effect than (E × T). Overall, comparing the F values of these various factors, exposure (E), mainly soil irrigation, displayed a more significant effect than (T) and (E × T) on the root mineral profile.

Neither treatment, exposure, nor their combined effect altered Co, Li, Mn, Pb, Sn, U, or V concentrations in leaves (Table 1) and were not represented in Fig. 4. To sum up, the influence of treatment was the main factor of most elements' variability in leaves, except for Cu. Overall, CuSO₄-treated pots presented more alterations on the leaf mineral profile than CuO NP-treated pots (Fig. 4). In leaves, Na, Mg, P, K, Ca, Cd, Cr, Zn, As, Se, Mo, Cd, and Ba concentrations were significantly affected only by (T) as follows: (i) Cr concentrations were significantly affected by CuO NP application; (ii) As, Mo, P, and Se concentrations were significantly affected by

CuSO₄ application and (iii) Na, K, Mg, Ca, Cd, Zn, and Ba were affected by both Cu forms (CuO NPs and CuSO₄); (iv) FeS and Ni in leaves were significantly affected by both (E) and (T) effects, mainly by both treatments (CuO NPs and CuSO₄) and a less significant effect by soil irrigation route of exposure was observed; (v) Cu, Ag, and Sb were significantly affected by (E), (T) and their interaction (E × T). Nevertheless, for Cu, the effect of foliar spray was the most pronounced factor in leaves, for Ag, it was the treatment effect (CuO NPs and CuSO₄), and for Sb, it was the CuSO₄ application.

Silver, Cu, Fe, and S had the greatest changes by both effects (T) and (E) among all the elements (Fig. 5a–d). This is an intriguing finding and merits further study to evaluate the processes involved in these elemental dynamics in “soil-plant system” under CuO NP exposure, because they are commonly associated in several geochemical reactions and minerals (Davis et al. 2001; Mantha et al. 2019; Stanley 1987) as well as in the biochemical reactions in roots and shoots (Dimkpa et al. 2012).

As the concentrations of Co, Li, Pb, Sn, and U in roots and leaves were statistically similar among the exposed groups and control, they were not discussed further in this study.

Macroelements (Ca, K, Mg, Na, P, and S)

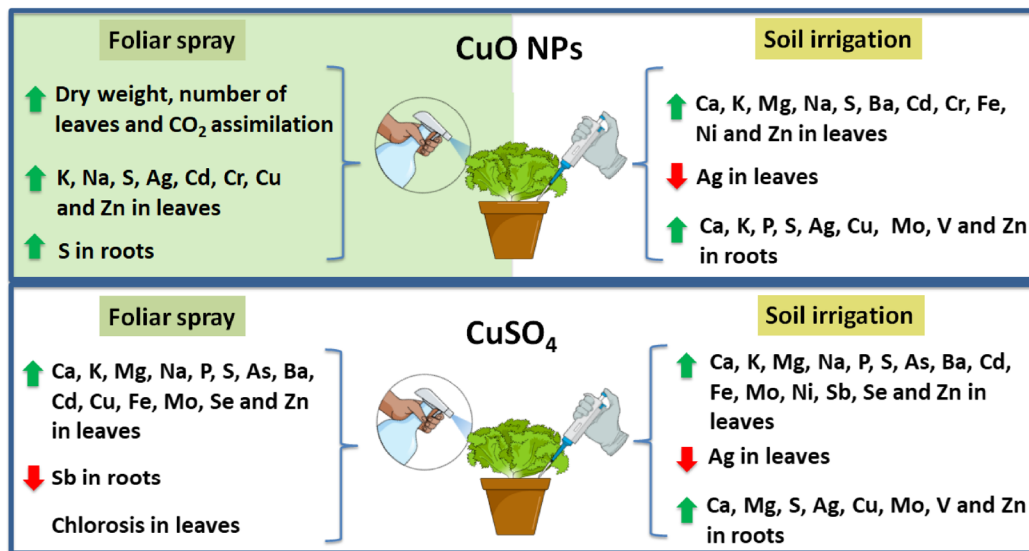
As previously described in Section 3.4.1, the elemental concentration in roots was mainly affected by the soil irrigation route of application. Sodium concentration in roots was not affected. Only the foliar exposure of CuO NPs caused a slight increase in S concentration in roots (19%). Calcium increased significantly in the roots (~ 20%) due to exposure by soil irrigation (NPs or salt). Nanoparticles caused no alteration in Mg content in roots and Cu salt soil irrigation increased it (30%). CuO NP exposure via soil irrigation caused a significant increase in P and K concentration in roots (36% and 27%, respectively) (Fig. 4).

Overall, the concentration of macroelements in leaves from the groups exposed to Cu (NPs or Salt) was higher than in the control samples (Fig. 4). No changes were observed for Ca and Mg concentrations in CuO NP-treated pots exposed by foliar spray and for P concentrations in CuO exposure compared to the control. In general, the increase in macroelement concentration in leaves was higher in CuSO₄-treated pots than in CuO NP-treated pots, regardless of the route of application. Comparing the two routes of application, the soil irrigation route caused a higher increase in the concentration of these macroelements.

Contrary to our findings, Hong et al. (2015) have found that the application of CuO NPs (< 100 nm) at 20 mg L⁻¹ in lettuce decreased P in roots and leaves. Cota-Ruiz et al. (2020) reported that P and S contents were reduced in bulk and ionic Cu-exposed (80 and 280 mg kg⁻¹) alfalfa plants compared to

Table 1 The elemental concentrations (dry base) in lettuce plant compartments (leaves and roots) after harvest by exposure and by treatment (mean \pm SD) ($n = 4$)

Sample	Exposure	Treatment	Ag ($\mu\text{g kg}^{-1}$)	As ($\mu\text{g kg}^{-1}$)	Ba (mg kg^{-1})	Ca (mg kg^{-1})	Cd ($\mu\text{g kg}^{-1}$)	Co ($\mu\text{g kg}^{-1}$)
Leaves	Control	Control	13 \pm 3	21 \pm 4	9 \pm 1	14317 \pm 2039	60 \pm 5	10 \pm 2
Leaves	Foliar spray	CuO NPs	17 \pm 3	23 \pm 4	10 \pm 1	16773 \pm 1518	70 \pm 9	10 \pm 2
Leaves	Soil irrigation	CuO NPs	6 \pm 3	25 \pm 4	12 \pm 3	22274 \pm 6871	75 \pm 13	10 \pm 2
Leaves	Foliar spray	Cu salt	4 \pm 1	27 \pm 5	13 \pm 1	21394 \pm 2715	70 \pm 8	9 \pm 1
Leaves	Soil irrigation	Cu salt	6 \pm 3	32 \pm 5	12 \pm 0.4	22751 \pm 250	71 \pm 5	13 \pm 3
Roots	Control	Control	66 \pm 2	426 \pm 87	23 \pm 6	8214 \pm 410	319 \pm 31	119 \pm 33
Roots	Foliar spray	CuO NPs	73 \pm 8	404 \pm 93	18 \pm 3	8877 \pm 598	322 \pm 27	110 \pm 11
Roots	Soil irrigation	CuO NPs	83 \pm 24	501 \pm 115	21 \pm 3	10079 \pm 573	388 \pm 94	149 \pm 10
Roots	Foliar spray	Cu salt	54 \pm 7	413 \pm 111	21 \pm 3	8547 \pm 162	297 \pm 37	122 \pm 9
Roots	Soil irrigation	Cu salt	90 \pm 10	510 \pm 54	21 \pm 3	9715 \pm 357	367 \pm 67	124 \pm 10
Sample	Exposure	Treatment	Mo (mg kg^{-1})	Na (mg kg^{-1})	Ni ($\mu\text{g kg}^{-1}$)	P (mg kg^{-1})	Pb ($\mu\text{g kg}^{-1}$)	S (mg kg^{-1})
Leaves	Control	Control	2.9 \pm 0.5	550 \pm 64	181 \pm 23	10313 \pm 216	75 \pm 10	3922 \pm 291
Leaves	Foliar spray	CuO NPs	3.6 \pm 0.3	699 \pm 12	200 \pm 27	11521 \pm 729	70 \pm 11	4620 \pm 513
Leaves	Soil irrigation	CuO NPs	5 \pm 2	764 \pm 102	311 \pm 93	12303 \pm 2682	69 \pm 12	5723 \pm 1075
Leaves	Foliar spray	Cu salt	4.7 \pm 0.5	887 \pm 60	180 \pm 9	12382 \pm 1066	68 \pm 7	5538 \pm 140
Leaves	Soil irrigation	Cu salt	4.6 \pm 0.4	934 \pm 57	254 \pm 31	13808 \pm 748	69 \pm 10	5956 \pm 199
Roots	Control	Control	9.8 \pm 0.5	2856 \pm 459	564 \pm 57	11486 \pm 1227	1056 \pm 147	3736 \pm 238
Roots	Foliar spray	CuO NPs	11 \pm 1	3840 \pm 960	544 \pm 73	11372 \pm 1611	808 \pm 184	4458 \pm 176
Roots	Soil irrigation	CuO NPs	13 \pm 1	3589 \pm 1664	562 \pm 115	15593 \pm 1544	1012 \pm 251	5079 \pm 466
Roots	Foliar spray	Cu salt	10 \pm 1	1971 \pm 256	450 \pm 48	12302 \pm 2478	863 \pm 244	4366 \pm 413
Roots	Soil irrigation	Cu salt	13 \pm 2	4755 \pm 2355	549 \pm 124	13841 \pm 1802	891 \pm 132	4841 \pm 366
Sample	Cr ($\mu\text{g kg}^{-1}$)	Cu (mg kg^{-1})	Fe (mg kg^{-1})	K (mg kg^{-1})	Li ($\mu\text{g kg}^{-1}$)	Mg (mg kg^{-1})	Mn (mg kg^{-1})	
Leaves	275 \pm 45	5.2 \pm 0.4	40.9 \pm 0.3	82859 \pm 4342	24 \pm 2	2987 \pm 451	9 \pm 1	
Leaves	435 \pm 32	45 \pm 7	43 \pm 6	106238 \pm 8890	28 \pm 7	3255 \pm 192	10 \pm 1	
Leaves	479 \pm 135	6 \pm 2	54 \pm 9	122181 \pm 22229	27 \pm 6	4169 \pm 1072	10 \pm 2	
Leaves	411 \pm 144	55 \pm 4	46 \pm 3	127755 \pm 9635	29 \pm 4	4049 \pm 482	10 \pm 1	
Leaves	413 \pm 26	6.5 \pm 0.2	52 \pm 4	137433 \pm 6199	31 \pm 3	4337 \pm 229	12 \pm 4	
Roots	1204 \pm 313	59 \pm 5	408 \pm 152	42499 \pm 1443	224 \pm 84	4465 \pm 210	10 \pm 2	
Roots	896 \pm 255	68 \pm 9	319 \pm 79	46353 \pm 6199	150 \pm 44	4709 \pm 378	8 \pm 1	
Roots	1101 \pm 276	91 \pm 27	396 \pm 91	54029 \pm 3191	188 \pm 38	4871 \pm 785	12 \pm 2	
Roots	1048 \pm 288	56 \pm 16	361 \pm 106	51465 \pm 6360	179 \pm 58	3931 \pm 241	10 \pm 2	
Roots	1071 \pm 215	89 \pm 22	405 \pm 89	52810 \pm 7598	192 \pm 47	5805 \pm 612	10 \pm 2	
Sample	Sb ($\mu\text{g kg}^{-1}$)	Se ($\mu\text{g kg}^{-1}$)	Sn ($\mu\text{g kg}^{-1}$)	U ($\mu\text{g kg}^{-1}$)	V ($\mu\text{g kg}^{-1}$)	Zn (mg kg^{-1})		
Leaves	4 \pm 1	49 \pm 19	12 \pm 2	0.9 \pm 0.1	42 \pm 11	22 \pm 1		
Leaves	3 \pm 1	43 \pm 10	14 \pm 2	0.8 \pm 0.2	41 \pm 15	25 \pm 1		
Leaves	4 \pm 1	72 \pm 13	14 \pm 3	0.96 \pm 0.50	61 \pm 20	27 \pm 5		
Leaves	4 \pm 1	96 \pm 14	13 \pm 1	0.8 \pm 0.3	49 \pm 8	25 \pm 2		
Leaves	8 \pm 3	87 \pm 29	14 \pm 1	0.8 \pm 0.4	59 \pm 7	27 \pm 1		
Roots	189 \pm 32	203 \pm 49	187 \pm 70	125 \pm 32	13160 \pm 1604	35 \pm 1		
Roots	163 \pm 27	180 \pm 35	129 \pm 14	108 \pm 24	14114 \pm 1704	38 \pm 2		
Roots	202 \pm 30	246 \pm 84	182 \pm 35	126 \pm 30	16766 \pm 2921	43 \pm 6		
Roots	141 \pm 15	262 \pm 39	150 \pm 20	102 \pm 7	12967 \pm 819	36 \pm 2		
Roots	175 \pm 29	258 \pm 48	119 \pm 28	133 \pm 42	16563 \pm 2619	42 \pm 4		



	Roots				Leaves				
	Element	Foliar Spray		Soil Irrigation		Foliar Spray		Soil Irrigation	
		NPs	Salt	NPs	Salt	NPs	Salt	NPs	Salt
Macro Elements	Ca	=	=	+23%	+18%	=	+49%	+55%	+59%
	K	=	=	+27%	=	+28%	+54%	+47%	+66%
	Mg	=	=	=	+30%	=	+40%	+36%	+45%
	Na	=	=	=	=	+27%	+61%	+39%	+70%
	P	=	=	+36%	=	=	+20%	=	+33%
	S	+19%	=	+36%	+30%	+18%	+41%	+46%	+52%
Trace Elements	Ag	=	=	+26%	+36%	+31%	-69%	-54%	-54%
	As	=	=	=	=	=	+29%	=	+52%
	Ba	=	=	=	=	=	+44%	+33%	+33%
	Cd	=	=	=	=	+17%	+17%	+25%	+18%
	Cr	=	=	=	=	+58%	=	+74%	=
	Cu	=	=	+54%	+51%	+765%	+957%	=	=
	Fe	=	=	=	=	=	+12%	+32%	+27%
	Mo	=	=	+33%	+33%	=	+62%	=	+59%
	Ni	=	=	=	=	=	=	+71%	+40%
	Sb	=	-25%	=	=	=	=	=	+100%
	Se	=	=	=	=	=	+96%	=	+78%
	V	=	=	+27%	+26%	=	=	=	=
	Zn	=	=	+23%	+20%	+13	+13	+22	+22

Fig. 4 Macro and trace elements results in roots and leaves of lettuce treated with different Cu compounds (NPs or salt) via two routes of exposure (foliar spray and soil irrigation). Mean significant increments compared to the control are signaled by (+) and reductions (-), and unaffected (equal symbol) ($n = 4$)

the controls. Copper NPs ($100\text{--}500\text{ mg kg}^{-1}$ of sand for 7 days) decreased the Ca and Mg contents in leaves of bean plants (Dimkpa et al. 2015) and Cu NPs (20 mg L^{-1} for 15 days) decreased concentrations of P, Ca, and Mg in lettuce (Trujillo-Reyes et al. 2014). The application of TiO_2 NPs ($50\text{--}400\text{ mg L}^{-1}$) in lettuce (*Lactuca sativa* L.) reduced the content of Ca (35.7%), Mg (27.2%), and Na (15.4%) compared to the control (Hu et al. 2020).

On the other hand, Hayes et al. (2020) evaluated the application of Al_2O_3 NPs in lettuce and reported increased concentrations of Mg, P, and Ca compared to the control. CuO NPs (150 mg kg^{-1}) on green onion increased root Ca (86%), bulb Ca (74%), and bulb Mg content in roots and leaves as well (108%) (Wang et al. 2020). However, at the same concentration, the salt application reduced Ca ($\sim 65\%$) and Mg ($\sim 30\%$) concentrations in roots (Wang et al. 2020).

Beyond the comparison among treatments and control, it was essential to evaluate if the nutrient content on leaves was suitable for lettuce plant nutrition. In comparison with the determined values: Mg ($2.99\text{--}4.34\text{ g kg}^{-1}$), S ($3.92\text{--}5.96\text{ g kg}^{-1}$), and Ca ($14.32\text{--}22.75\text{ g kg}^{-1}$) in the leaves, these elements are within the range recommended by Malavolta

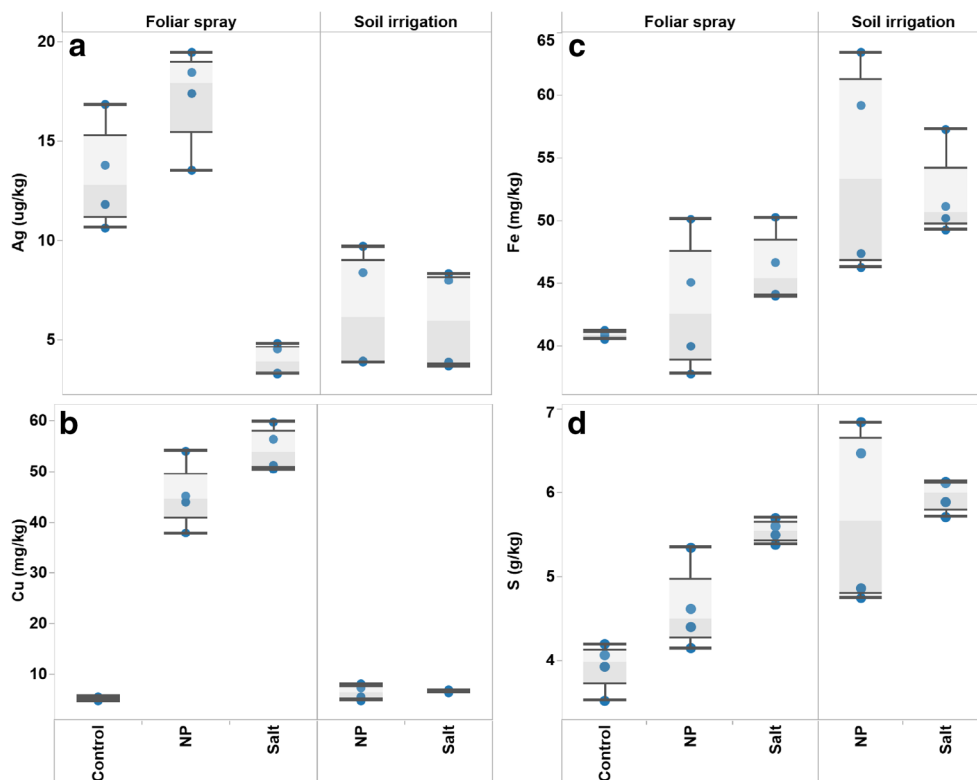
(1980), Pais and Jones Jr (1997), and Furlani (2004) as follows: Mg ($3\text{--}5\text{ g kg}^{-1}$), S ($1\text{--}5\text{ g kg}^{-1}$), and Ca ($10\text{--}50\text{ g kg}^{-1}$). However, P ($10.31\text{--}13.81\text{ g kg}^{-1}$) and K ($82.86\text{--}137.43\text{ g kg}^{-1}$) were higher than the recommended values by these authors, P ($1\text{--}3\text{ g kg}^{-1}$), K ($8\text{--}50\text{ g kg}^{-1}$), but also in the control samples, most probably due to the high levels of these elements in the soil composition of the urban garden (Table S1).

Trace elements (Ag, As, Ba, Cd, Cr, Cu, Fe, Mo, Ni, Sb, Se, V, and Zn)

As expected, Cu concentration increased in leaves after the application of CuO NPs and CuSO_4 (Fig. 5b) compared to the control. As mentioned before, this increase was more intense with foliar spray than soil irrigation for both materials (CuO NPs and CuSO_4). Foliar Cu accumulation in leaves was similar regardless of the Cu form applied (nano $45 \pm 7\text{ mg kg}^{-1}$ or salt $55 \pm 4\text{ mg kg}^{-1}$). The accumulated Cu was mostly inside the tissues, since the lettuces were washed thoroughly; the same was observed by Zhao et al. (2016a) after they exposed lettuce to $\text{Cu}(\text{OH})_2$ nanopesticide by foliar spray.

According to Anjum et al. (2015), levels of $20\text{--}30\text{ mg Cu kg}^{-1}$ in leaves (DW) were considered toxic for most crop species. However, no chlorosis spots or loss of productivity were observed for lettuce heads from CuO NPs by foliar spray exposure. This result may be positive for plant development, since Cu homeostasis is essential for proper plant growth because it is important for the cuproproteins cofactor. Zhao et al.

Fig. 5 Concentrations of Ag, Cu, Fe, and S measured in lettuce leaves by exposition and treatment. These elements were significantly influenced by the effect of exposition and treatment ($n = 4$)



(2016c) observed that after the exposure of lettuce leaves to foliar applications of $\text{Cu}(\text{OH})_2$ nanopesticides, even at high concentrations of Cu ($823\text{--}2501 \text{ mg kg}^{-1}$), the lettuce leaves did not present any visible toxic symptoms throughout the entire exposure period. In our study, chlorosis spots were observed on leaves after CuSO_4 foliar spray application. This may occur due to the affinity of ionic copper to complex with sulfhydryl groups present in amino acids or antioxidants such as reduced glutathione (GSH), and these complexes are phytotoxic, being involved in GSH oxidation via interaction with thiol groups (Dimkpa et al. 2012).

None of the soil irrigation groups (nano $6.0 \pm 2.0 \text{ mg kg}^{-1}$ or salt $6.5 \pm 0.2 \text{ mg kg}^{-1}$) promoted any considerable increase on the Cu accumulation in leaf tissues compared with the controls ($5.2 \pm 0.4 \text{ mg kg}^{-1}$) and the foliar exposure produced 8-fold more Cu in leaves than soil irrigation. The results observed for exposure via soil irrigation may be attributed to the fact that the soil used for the pot experiment was slightly acidic. In this kind of environment, CuO NPs are dissolved and act similarly to dissolved Cu; in the long term, they can transform into Cu bound to $\text{FeO}(\text{OH})$ or OM transforms into Cu_2S (Peng et al. 2017; Sekine et al. 2017).

Similar results were observed by Servin et al. (2017). They evaluated the bioaccumulation of CuO in NPs, bulk, and ionic form ($0\text{--}400 \text{ mg kg}^{-1}$) added into soil, by lettuce exposed for 70 days. The soil used by these authors (sandy loam, 69% sand, 22% silt, 8.6% clay, 4.3% OM, pH 5.9, CEC 186 mmol dm^{-3}) was very similar to the soil used in this present study and they observed concentrations of Cu from 7.0 ± 3.0 (control-unexposed) to $9.0 \pm 1.7 \text{ mg kg}^{-1}$ (NPs) and $9.0 \pm 1.6 \text{ mg kg}^{-1}$ (ion). The Cu concentrations added in soil by Servin et al. (2017) were much higher than ours and this may indicate that lettuce exposed to Cu in soil, regardless of the form (nano or salt) has a maximum capacity to accumulate Cu in leaves. Early studies have reported that Cu translocation to edible parts of lettuce seemed to be well regulated, as their concentrations were fairly constant (Ginocchio et al. 2002).

In this study, root concentrations of Cu varied widely, especially in samples of soil irrigation treatments from 54 to 64 mg kg^{-1} (control), 64 to 118 mg kg^{-1} (CuO NPs), and 67 to 111 mg kg^{-1} (CuSO_4) (Table 1). The Cu translocation from roots to leaves for soil irrigation treatments (NPs or salt) was similar to the control samples (7 to 8%), which means that the accumulation of Cu in roots was around 92%. Previously, other studies reported Cu accumulation rates ranging from 87 to 99% in the roots, with very low translocation to leaves for lettuce, alfalfa, and cilantro (Hong et al. 2015; Servin et al. 2017; Zuverza-Mena et al. 2015).

According to Fig. 4, foliar exposure of Cu (NPs or salt) did not cause a disturbance in trace element concentrations in roots, except for Sb, in which the concentration was reduced in CuSO_4 -treated pots. Soil irrigation exposure, regardless of the treatment (NPs or salt), increased the concentrations of Ag,

Mo, V, and Zn (from 20 to 36%) in roots compared to the control.

In leaves, after soil irrigation, Ag content was significantly smaller (54%) than the control when NPs and Cu salt were applied (Fig. 5a). On the other hand, Ag increased with the foliar NP application (31%) and decreased (69%) by the foliar salt application when compared to the levels of the control samples. This is an interesting finding, since the accumulation of Ag in plants exposed to CuO NPs was very distinct depending on the route of application. The Ag translocation mechanism is most probably associated with the same processes of free Cu translocation, and the lettuce mechanism to accumulate free Cu in roots also probably triggers Ag accumulation in roots. Copper and Ag have similar geochemical behavior. Silver rarely occurs in nature in a soluble form; thus, available Ag for plant uptake is restricted (Nguyen et al. 2017). According to Settimio et al. (2014), the labile forms of Ag in soil fractions are Ag^+ , reversibly sorbed Ag^+ to Fe-oxohydroxides and organic S of OM; Ag^+ weakly complexed with other soil solution ligands (L); the non-labile Ag is solid: metallic Ag, AgCl, and Ag_2S ; and Ag irreversibly bound to organic S and Fe-oxohydroxides (surface precipitated or fixed within crystal lattices). Excess of free Cu is known to stimulate the release of amino acids, carboxyl and amino groups, organic acids, glutathione, cysteine, to complex, or chelate with Cu with other root exudates that can bind and reduce the availability of Cu and Ag to leaves (Zhao et al. 2016b).

In leaves (Fig. 4), there was an increase in Cd and Zn concentrations in all pot groups compared to the control (from 13 to 25%). CuO NP-treated pots exposed by foliar spray caused an increase in Cd, Cr, and Zn levels in leaves. Arsenic, Mo, and Se concentrations in leaves were increased only in the CuSO_4 -treated pots by both routes of exposure. This is noteworthy because these three elements can commonly exist as oxyanions in soil-oxidizing environments. Soil irrigation exposure caused an increase in Ni (71% NPs and 40% salt) and an increase in Sb in leaves (100%), only for CuSO_4 -treated pots. Iron content in leaves increased in almost all pot groups, except in CuO NP-treated pots exposed by foliar spray.

The soil collected from an urban garden used for our pot experiment presented a high level of Ba (Table S1), a fact also observed in soils from other urban gardens (McBride et al. 2014; Nabulo et al. 2010). Thus, an undesirable effect of CuO NP exposure in our study would be a higher accumulation of Ba in leaves. Despite that, Ba content in leaves increased in almost all pot groups, except in CuO NP-treated pots exposed by foliar spray. Nevertheless, the range of concentration found in our study (9 to 13 mg kg^{-1}) for this element is in good agreement with the mean value (12 mg kg^{-1}) obtained for leafy green vegetables purchased in New York supermarkets (McBride et al. 2014).

Opposite findings were reported regarding the role of NPs on Cr uptake in plants. Magnetite NPs (López-Luna et al. 2016) and biogenic CuNPs (Noman et al. 2020b) were found to reduce the translocation rate of this element from root to shoots. Also, similarly to our data, Noman et al. (2020a) reported a decreased Cd translocation by 49.62 % in wheat plants exposed to CuNPs added into soil. John (1976) claims that if the K level in plants is high, this could inhibit the Cd in leaves. In our study, the highest level of Cd in leaves was observed for foliar NP treatment, which presented the lowest level of K in leaves (Table 1).

Very scarce information about the effect of NPs on Mo accumulation in plants is available. Martínez-Fernández et al. (2016) exposed *Helianthus annuus* L. to Fe oxide NPs by hydroponic culture solution, and the concentration of Mo in the roots increased with the dose of NPs. In another study, with bell pepper (*Capsicum annum* L.) plants exposed to CuO NPs and CuCl₂ exposure, Mo uptake and transport of ionic copper treatment was probably supported by the P transporters (Rawat et al. 2018). The uptake, metabolism, and enzymatic mechanisms for Mo and Fe affect each other (Bittner 2014), and Mo is said to share some transporters with S and P (Nie et al. 2014). In our study, these four elements (Mo, Fe, P, and S) presented a similar pattern of accumulation, especially for the soil irrigation group (Table 1).

Estimated daily intake of chemical elements from lettuce

Since lettuce is one of the most acquired and consumed vegetables in Brazil and worldwide (Canella et al. 2018; Kim

et al. 2016), it is important to assess the contribution of the applied treatments/exposure to reach the dietary reference intakes (DRIs) of nutrients, as well as its influence on the intake of PTEs. Table 2 shows this information for some elements that presented significantly differences among pot groups and available information about dietary reference dose according to international regulations.

For all elements except Cu, small differences were observed in the EDI between pot groups (less than twice from one group to another). However, the foliar application of salt increased Cu EDI about 10 times in the control, changing from 2.30 to 24.2% of the supplied percentage of the DRI of this element. The EDI for Cu ranged from 0.021 (control) to 0.181 mg day⁻¹ (NPs via foliar application) and 0.218 mg day⁻¹ (salt via foliar application), which is within recommended intake guidelines of 0.9 mg Cu/ person-day, thus safe for human consumption. Higher values were reported by Zhao et al. (2016a). These authors investigated the impact of Cu(OH)₂ nanopesticide on the nutritional value of lettuce and observed a considerable increase in the EDI of Cu with exposure; the leaves accumulated around 1350–2010 mg Cu kg⁻¹ DW after 30 days of foliar exposure. The EDI reported by them was 0.021 mg day⁻¹ for the control, 2.2 mg day⁻¹ for the low level of exposure, and 3.3 mg day⁻¹ for the high level (Zhao et al. 2016a).

For Fe, Na, and Zn, the EDI did not represent a considerable percentage of the DRI in any pot group (Table 2). On the other hand, for Mg, P, and Ca, the consumption of lettuce can contribute with 2.84 to 9.58% of the DRI. Moreover, the ingestion can contribute to 9.75 to 21.1% of the DRI for K, so

Table 2 Estimated daily intake (EDI) of chemical elements through the consumption of lettuce submitted to different treatments

Estimated daily intake						
Mean intake (mg day ⁻¹) (% of the DRI or PTDI)						
Element	Control	Foliar spray/NP	Soil irrigation/NP	Foliar spray/salt	Soil irrigation/salt	DRI ^a /PTDI ^b
Ca	57.3 (4.77–5.73)	67.1 (5.59–6.71)	89.1 (7.42–8.91)	85.6 (7.13–8.56)	91.0 (7.58–9.10)	1000–1200 ^a
Cd	0.000241 (0.96)	0.000279 (1.12)	0.000299 (1.20)	0.000280 (1.12)	0.000285 (1.14)	0.025 ^b
Cr	0.00110 (3.14–5.49)	0.00174 (4.97–8.70)	0.00192 (5.47–9.58)	0.00164 (4.70–8.22)	0.00165 (4.72–8.26)	0.020–0.035 ^a
Cu	0.021 (2.30)	0.181 (20.1)	0.026 (2.83)	0.218 (24.2)	0.026 (2.90)	0.900 ^a
Fe	0.164 (0.908–2.04)	0.173 (0.960–2.16)	0.216 (1.20–2.70)	0.185 (1.03–2.31)	0.208 (1.15–2.60)	8.00–18.0 ^a
K	331 (9.75–12.7)	425 (12.5–16.3)	489 (14.4–18.8)	511 (15.0–19.7)	550 (16.2–21.1)	2600–3400 ^a
Mg	11.9 (2.84–3.85)	13.0 (3.10–4.20)	16.7 (3.97–5.38)	16.2 (3.86–5.22)	17.3 (4.13–5.60)	310–420 ^a
Mo	0.0118 (26.2)	0.0146 (32.4)	0.0203 (45.1)	0.0187 (41.5)	0.0182 (40.5)	0.045 ^a
Na	2.20 (0.147)	2.80 (0.186)	3.06 (0.204)	3.55 (0.236)	3.73 (0.249)	1500 ^a
P	41.3 (5.89)	46.1 (6.58)	49.2 (7.03)	49.5 (7.08)	55.2 (7.89)	700 ^a
Zn	0.089 (0.812–1.12)	0.098 (0.893–1.23)	0.109 (0.993–1.37)	0.101 (0.919–1.26)	0.109 (0.990–1.36)	8.00–11.0 ^a

^a Dietary Reference Intakes (DRIs) englobes the Recommended Dietary Allowances and Adequate Intakes for essential elements established by the Food and Nutrition Board, National Academies (Del Valle et al. 2011; Institute of Medicine 1997, 2001; National Academies of Sciences, Engineering, and Medicine 2019). ^b Provisional Tolerable Daily Intake (PTDI) for toxic elements, based on Tolerable Weekly Intake (TWI)/7 (EFSA 2011). Considering a 70-kg body weight person

lettuce can be a good source of K. The salt application by soil irrigation provided the highest EDI values for Na, Mg, P, K, and Ca, while for Zn, both treatments provided the same EDI (Table 2). Thus, CuO NP exposure for both routes of application in lettuce did not cause a considerable change (less than 1.5-fold compared to the control) on Na, Ca, Zn, Mg, K, and P supply. Moreover, Kemi et al. (2010) suggested that the Ca/P ratio in food must be 1.0 and that a low dietary Ca/P ratio may interfere with the homeostasis of Ca metabolism in humans. Our results showed that the Ca/P ratio was higher than 1 for all treatments ranging from 1.4 (control) to 1.8 (salt foliar exposure).

For Cd, the treatments did not affect EDI significantly. The percentages of the provisional tolerable daily intake (PTDI) provided varied from 0.96 to 1.20%, which is very low. Fortunately, the consumption of lettuce obtained in these pot trials did not pose a risk to consumers due to Cr ingestion, since the highest EDI (0.00192 NPs exposure by soil irrigation) represents 5.47 to 9.58% of the DRI for Cr (Table 2). On average, the EDI values observed in our study are similar to the value reported (0.00144) by Pillay and Jonnalagadda (2007) for lettuce from various supermarkets in Durban (South Africa).

Conclusion

Green tea-synthesized CuO NPs were found to have spherical shape and small size. Lettuce plants were exposed in a pot experiment to fixed concentrations of copper, as CuO NPs and CuSO₄ by foliar spray, and soil irrigation as application routes. For lettuce, exposure via foliar application improved the number of leaves and DW and caused a slight improvement in gas exchange. Moreover, all the lettuce exposed to copper nano or salt form showed changes in elemental accumulation in plant tissues (leaves and roots), mainly increasing their concentration in roots and leaves. However, foliar application of NPs was the one that resulted in less disturbance of lettuce mineral profile. Copper salt exposure resulted in a more aggressive alteration of the mineral profile compared to the control, regardless of the route of application, and caused necroses in leaves by foliar spray exposure. CuO NPs application via foliar spray caused accumulation of K, Na, S, Ag, Cd, Cr, Cu, and Zn in leaves. Nevertheless, this increase was not of concern to consumers. Further studies are required to evaluate the impact of these CuO NPs under field conditions and their fate in the environment. Also, it is strongly recommended that the effects on enzymatic, molecular, and genetic levels of lettuce plants be assessed, but these topics are beyond the scope of this study.

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Data availability All data supporting the conclusions of this article are provided as figures, tables, and supplementary tables and figures.

Compliance with ethical standards

Ethics approval and consent to participate Not applicable.

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