Science of the Total Environment 618 (2018) 306–312

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Differential effects of copper nanoparticles/microparticles in agronomic and physiological parameters of oregano (*Origanum vulgare*)

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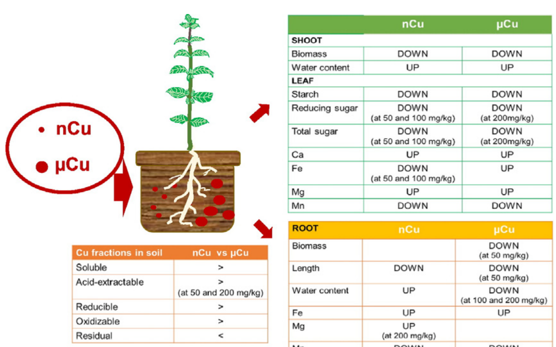
HIGHLIGHTS

- Soluble Cu content increased with applied μCu, but not with nCu.
- μCu showed more inhibiting effect on oregano root growth than nCu.
- Both nCu and μCu decreased leaf starch, total sugar, and reducing sugar in oregano.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 August 2017
Received in revised form 3 November 2017
Accepted 3 November 2017
Available online xxxx

Editor: D. Barcelo

Keywords:
Copper
Nanoparticle
Oregano
Phytotoxicity
Microparticle

ABSTRACT

The effects of metallic copper nanoparticles (nCu) in plants are not well understood. In this study, soil grown oregano (*Origanum vulgare*) was exposed for 60 days to nCu and Cu microparticles (μCu) at 0–200 mg Cu/kg. At harvest, Cu accumulation, biomass production, nutrient composition, and Cu fractions in soil were measured. Except for μCu at 50 mg/kg, both nCu and μCu increased root Cu (28.4–116.0%) and shoot Cu (83.0–163.0% and 225.4–652.5%, respectively), compared with control. Copper accumulation from μCu increased as the external μCu increased. nCu and μCu did not affect shoot length, malondialdehyde, or chlorophyll, but increased water content (6.9–12.5%) and reduced shoot biomass (21.6–58.5%), compared with control. In addition, at 50 mg/kg, μCu decreased root biomass and length (48.6% and 20.5%, respectively) and water content (1.8% and 3.9% at 100 and 200 mg/kg, respectively). All treatments modified root and shoot Ca, Fe, Mg, and Mn ($p \le 0.05$). Additionally, all Cu treatments decreased starch (33.9–58.5%), total sugar (39.5–55.7%), and reducing sugar (13.6–33.9%) in leaves. Results showed that metallic Cu nanoparticles/microparticles affected agronomical and physiological parameters in oregano, which could impact human nutrition. However, smaller size particles do not necessarily imply greater toxicity.

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https://doi.org/10.1016/j.scitotenv.2017.11.042
0048-9697/

1. Introduction

Nanoparticles are considered to impact the environment in a different way than their corresponding microsize particles, due to their higher surface area to volume ratio, and chemical reactivity (Hong et al., 2014; Morales et al., 2013). Available data suggest that the bioactivity and toxicity of some nanoparticles and microparticles is correlated with the amount of released ions (Dimkpa et al., 2013; Musante and White, 2012; Zuverza-Mena et al., 2015).

Copper nanoparticles (nCu) are intensively applied in a variety of fields including facial sprays, additives in lubricants, metallic coating and inks, and anode materials for lithium ion batteries (Song et al., 2014). They can be used as pesticides, fungicides, and as fertilizers to deliver the micronutrient Cu to plants (Liu and Lal, 2015; Tegenaw et al., 2015). Hence, they can enter into agroecosystems, leading to negative impacts on soil-plant systems (Gao et al., 2017; Lee et al., 2008). Very few studies have reported the effects of nCu to terrestrial plants. The data indicate that they affect plant growth, biomass production, and plant transpiration (Lee et al., 2008; Musante and White, 2012; Stampoulis et al., 2009). The time and aging with regard to the fate and bioavailability of Cu compounds have also been documented (Brennan et al., 1980; Gao et al., 2017; Ma et al., 2006). However, the size-specific effects for Cu have not been well elucidated. Most studies have failed to directly compare bulk and nanoparticulate behavior for a given material (Musante and White, 2012). It is, therefore, necessary to understand whether the effects of nCu are different from those of Cu microparticles (μCu) in order to assess the impact of nCu to agroecosystems.

Oregano (*Origanum vulgare*) is a culinary herb consumed fresh worldwide, especially in South European cuisine and Latin America. The leaves, dried herbs, as well as the volatile oil of this aromatic perennial herb have been used medicinally for centuries (Chun et al., 2005). As many herb plants, oregano is affected by pest and diseases. In California, USA, oregano is infested by *Puccinia menthae*, which significantly reduces the quality and yield of this culinary herb (Koike et al., 1998). In Italy, *Phytophthora tentaculata* (Kröber & Marwitz) produces root and basal stem rot in oregano, tarnishing about 20% of the stock of potting plants (Martini et al., 2009). In addition, Greek and Turkish oregano are attacked by a leafhopper that reduces the essential oil content by up to 34.8% (Arslan et al., 2012). Copper-based nanoparticles have shown potential as pesticides (Kanhed et al., 2014), which suggests that they can be sued to control fungal diseases in oregano. In this study, copper fractionation in soil, biomass production, content of chlorophylls, malondialdehyde, water, as well essential elements, and macromolecules were evaluated in fully grown oregano. The effects of nCu on agronomic and physiological parameters were compared with those of the corresponding bulk material, μCu.

2. Materials and methods

2.1. Characterization of nCu and μCu

Copper nanoparticles and microparticles (Sigma Aldrich Co., St. Louis, MO) have been previously characterized in solid and suspension forms (Adeleye et al., 2014; Hong et al., 2015; Zuverza-Mena et al., 2015). Primary size, hydrodynamic diameter, zeta potential, and other physicochemical characteristics are shown in Table S1.

2.2. Preparation of suspensions/solutions and soil

Copper nanoparticles/microparticles suspensions/solutions were prepared in Millipore water and homogenized by sonication in a water bath (Crest Ultrasonics, Trenton, NJ) for 30 min at 10 °C with a sonication intensity of 180 watts. Subsequently, enough volume of each suspension was thoroughly mixed with 2 kg (wet weight, 902.8 dry weight, 54.9% water) commercial potting mix (Miracle Gro® with micromax, Marysville, OH, USA) to have 0 (control), 50, 100 and 200 mg Cu per kg soil (dry weight). The spiked soil was placed in plastic pots (19 cm diameter × 18 cm height) and seeded 24 h after treatment application. Four replicates for each treatment were allocated in a random design.

2.3. Plant growth and harvest

Oregano seeds were purchased from Mountain Valley Seed, Utah, USA. Forty seeds per pot were planted at 1 cm depth from the surface and placed into the growth chamber (Environmental Growth Chamber, Chagrin Falls, OH, USA) at temperature of 25/20 °C day/night, 65% relative humidity, light intensity of 340 $\mu mol\ m^{-2}\ s^{-1}$ and a 14 h photoperiod. Ten days after germination, plants were thinned to 20 plants per pot. Control and Cu exposed plants were watered with tap water (no Cu detected) every day and no fertilizer was added. Sixty days after germination, plants were removed from the soil, rinsed with tap water to remove excess soil, soaked in 0.01 M HNO_3 for about 10 s, and rinsed three times with Millipore water.

2.4. Copper sequential extraction of soil

After plant harvest, soil was collected and analyzed for soluble copper, according to Dimkpa et al. (2015). Copper fractionation in soil was determined by sequential extraction using the method of the Commission of the European Communities (Community Bureau of Reference, BCR). The method is described in detail in Quevauviller et al. (1997).

2.5. Chlorophyll, malondialdehyde, and water content analysis

Contents of chlorophyll and malondialdehyde (MDA) were determined in fresh plants at harvest. Chlorophyll was extracted using 80% acetone and measured at 470, 646, and 663 nm according to Lichtenthaler and Welburn (1983). MDA was measured by monitoring thiobarbituric acid reactive substances at 459, 532, and 600 nm, as per Ohkawa et al. (1979). The fresh and dry weights of harvested oregano were used to calculate the water content (fresh weight – dry weight) and dry biomass (dry weight).

2.6. Element and macromolecule contents of harvested oregano

For elemental analysis, samples of 100 mg of oven dried tissues were digested with 6 ml of plasma pure HNO_3 (SPC Science, Champlain, NY). Digested samples were adjusted to 50 ml with Millipore water. Digests were analyzed for Cu, Ca, Fe, Mg, Mn, and Zn by inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin-Elmer Optima 4300 DV, Shelton, CT). Total sugar content was quantified from the glucose standard calibration curve at 490 nm, as per Dubois et al. (1956). Reducing sugar content was done at 620 nm according to Nelson-Somogyi (1952). Starch content was determined using residue from sugar extraction and same method as total sugar according to Verma and Dubey (2001).

2.7. Statistical analyses

The data were expressed as mean ± standard deviation ($n = 4$). Statistical significance of differences among treatments were determined using one-way analysis of variance and covariance (ANOVA), followed by Tukey's pair-wise comparisons at a significance level of 0.05.

3. Results and discussion

3.1. Copper fractionation in soil

Copper fractions in soil after 60 days are shown in Table 1. Although all Cu nanoparticles and microparticles underwent dissolution releasing soluble copper, only 1.1–2.4% and 0.5–0.8% of total Cu applied was found