Synthesis and Characterization of CuO Nanoparticles and Their Effect in Alfalfa Plants.

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Nanomaterials can improve the use efficiency of both nutrients and pesticides, decreasing environmental damage and reducing embodied energy losses [1]. However, a wide number of studies have demonstrated nanoparticles toxicity to plant species [2]. Among these, CuO nanoparticles (CuO-NPs) has been proved in crops like alfalfa and maize, where the seedlings showed a decrease in root and shoot growth. It is important to note, that the size distribution of the nanoparticles in these studies was heterogeneous around 20-200 nm and the concentrations were from 10 to 1000 ppm [2-4]. To our knowledge, there are no reports were the size of the CuO-NPs is less than 10 nm and their consequence in plants. The motivation of this work is to describe the structure and microstructural characteristics of CuO-NPs with an average particle size shorter than 10 nm and evaluate their effect in alfalfa plants.

CuO-NPs were obtained by the simple precipitation method [5], keeping a pH around 6.6-6.8 to precipitate the NPs. The structural characterization of CuO-NPs was performed by the x-ray diffraction (XRD) technique considering the Bragg Brentano geometry. The XRD patterns were collected on a PANalytical XPert PRO diffractometer equipped with an X´Celerator detector, using a Cu K α (λ = 1.5406 Å) monochromatic radiation and they were recorded in an interval of 2Θ = 10°-90° using a step-scanning of 0.02° with a counting time of 60 s. The CuO-NPs phase identification was performed using the Match program [6]. Transmission electron (TEM, Hitachi HT 7700) and field emission scanning electron (FE-SEM, JSM-7401F) microscopies were used to monitor the morphology features as particle size and shape distribution. The chemical composition was monitored by energy dispersive spectroscopy EDS analysis (Oxford Instruments). The sample´s preparation for TEM and FE-SEM comprises dispersed nanoparticles in ethanol at 10 mg/L. The particle size distribution was determined using the Image J software [7]. Selected area electron diffraction (SAED) complemented the structural characterization. Alfalfa plants were sprinkled (0.01 mL/cm³) with 200 ppm at two and four weeks of growth, and they were maintained at room temperature for 5 weeks. Finally, at six weeks of growth, we evaluated their growth and weight.

The CuO-NPs XRD patterns (Fig. 1a) shows the presence of reflections related to tenorite-type structure in the monoclinic phase and C 2/c space group indexed thorough the inorganic crystal card No. 80-1268. CuO-NPs TEM micrograph (Fig. 1b) reveals a quasi-spherical particle shape with a tendency to form agglomerates. SEM image (Fig. 1c) confirms the uniformity of the agglomeration of quasi-spherical particles with an average particle size between 4-5 nm (Fig. 1d). The EDS quantitative analysis (Fig. 1e) shows 38.43 % of oxygen and 26.75 % of Cu. The SAED ring pattern (Fig. 1f) indicates the polycrystalline nature of the samples and it was indexed in the monoclinic phase according to the inorganic crystal card No. 80-1268. In alfalfa plants, it was observed that the CuO-NPs increase the roots and shoots lengths in comparison with the control plants (Fig. 2a), also, the dry weight was increased (Fig. 2b). These results showed that the CuO-NPs could have a positive effect on plants, even when the concentration of CuO-NPs was ten times higher than in previous reports [3]. However, it is important to note that this effect could be due to the size of the nanoparticle as well as the culture conditions and more studies need to be done.



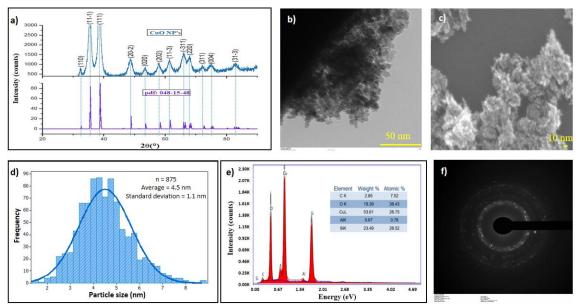


Figure 1. a) XRD pattern for CuO NPs. b) a) Bright field TEM image c) SEM micrograph. d) Nanoparticle size distribution and the Gaussian fitting. e) EDS elemental composition. f) SAED pattern for CuO NPs.

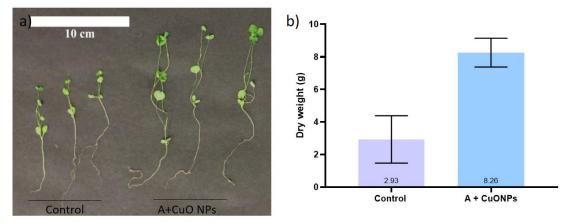


Figure 2. a) Representative image of alfalfa plants after six weeks of growth. Control: plants sprinkled only with water and A+CuO NPs: plants sprinkled with CuO NPs. b) Dry weight of alfalfa plants. Data were analyzed using t test.

References

- [1] GV Lowry, A Avellan, and LM Gilbertson, Nat. nanotechnol. 14 (2019), 517-522.
- [2] Z Wang, X Xie, J Zhao, X Liu, W Feng, J White, and B Xing, Environ. Sci Technol. 46 (2012) 4434-4441.
- [3] J Hong, CM Rico, L Zhao, AS Adeleye, AA Keller, JR Peralta-Videa, and JL Gardea-Torresdey, Environ. Sci. Proc. Imp. 17 (2014) 177-185.
- [4] MVJ Da Costa, and PK Sharma. Photosynthetica. 54 (2016) 110-119.
- [5] AS Lange, SJ Sharma, RB Pode, and RS Ningthoujam, Adv. Appl. Sci. Res. 1 (2010) 36-40.
- [6] H Putz, and K Brandenburg Match! Phase Identification from Powder Diffraction.
- [7] CA Schneider, WS Rasband, and KW Eliceiri, Nat. Methods. 9 (2012) 671-675.
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