Research Article

# Synthesis and Characterization of CuO Nanoparticles and Acute Lethal Studies in *Eudrilus eugeniae* Earthworm

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## ABSTRACT

**Introduction:** Metal oxide nanoparticles are widely used in numerous fields due to their wide interacting properties. Copperoxide nanoparticles (CuO NPs) has various application in industrial, agriculture, bioremediation and pharmaceutical. **Aim:** Our study is aimed to chemically synthesize the CuO NPs and revealed acute toxicity on earthworm *Eudrilus eugeniae*. **Materials and Methods:** CuO NPs are chemically synthesized by simple precipitation method. Nanoparticles characterization are studied by UV, FTIR, XRD, FESEM with EDAX. The acute toxicity of the CuO NPs on earthworm *Eudrilus eugeniae* are studied. **Results:** UV-Vis and FTIR spectroscopic studies revealed the gap energies and the functional group of the synthesized particles. XRD, FESEM with EDAX also evidenced the morphology of CuO NPs as spherical structure with 24nm size. The acute toxicity of the different concentrations CuO NPs are tested on acclimatized earthworm *Eudrilus eugeniae* (*n*=20) for 14 days. The lethal concentration ($LC_{50}$) of the CuO NPs on adult earthworm *E. eugeniae*is identified between 1100 to 1200ppm. **Conclusion:** The chemically synthesized CuO NPs confirmed particle size and nature. The emergence of CuO NPs into the soil medium becomes toxic to the earthworm.

**Keywords:** Copperoxide nanoparticles, SEM, $LC_{50}$, FTIR, Earthworm, *Eudrilus eugeniae*.

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## INTRODUCTION

Nanotoxicology is a rising revolutionary branch of science to deals with the toxicity of the nanomaterials as nanopowder and nanotubes which are widely used in agriculture, pharmaceutical and industrial applications. The emergence of nanomaterials into the environment pose a serious threat to the various non-targeted species. Nanoparticles (NPs) are ranged between 1-100nm. Nanoparticles has an unique chemical nature with remarkable surface chemistry.[1,2]

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When compared to the bulk materials, the respective metal oxide NPs develops more toxicity and bioaccumulation due to the high reactive properties. Nanoparticles synthesis and utilization for various anthropogenic activities leads to the outbreaks of these nanoparticles either accidently or intentionally or remains which eventually affects flora and fauna.[3-6]

Copper oxide NPs are widely used in gas sensors, catalyst, antibacterial and antifungal agents, textiles, water purifier, stable material for thermal and optical labile compounds, cosmetics, photovoltaic cells and coatings.[7-13] Due to this broad utilization, the release of CuO NPs into the ecosystem and biological exposure are significantly enhanced.

For the nanotoxicity studies, earthworms are considered as an ultimate invertebrate experimental species. Earthworms are generally found in all the soil types and also easy to handle in large quantities. Nanoparticles

entered into their system either through soil exposure or ingestion of contaminated soil or agricultural products. In a terrestrial ecosystem, earthworm functional disturbances will greatly influence the soil properties.[14-20] The objective of the study is to synthesize the metal oxide (CuO) nanoparticles by suitable method. Also to evidence the nature of the synthesized particles by various characterization studies such as FTIR, UV, XRD, FESEM with EDAX and to identify the toxicity of the CuO NPs on the earthworm, *Eudrilus eugenie* by probit analysis.

## MATERIALS AND METHODS

The Copperoxide nanoparticles are chemically synthesized with definite properties which are essential for the ecotoxicological studies. Nanoparticles characterization confirmed the synthesized NPs nature and acute toxicity analysis essential for the lethality of the CuO NPs exposure.

### Nanoparticle synthesis

Based on simple aqueous precipitation method, the metallic copper oxide (CuO) nanoparticles are synthesized. 2.9 gm of Copper (II) nitratetrihydrtate ($Cu(NO_3)_2$ .$3H_2O$) and1.2gm of Polyvinylpyrrolidone (PVP) were solubilized in 100ml deionized water. The solution is kept in the magnetic stirrer and heat the solution till it reaches 60°C. Simultaneously, 4% NaOH was prepared and added to the solution. Solution was constantly stirred at 60°C until the formation of black precipitation. Through Whatmann (No.1) filter paper, the solution are filtered. After discarding supernatant, the black pellets are collected.[21] Black pellets washed with distilled water and ethanol for 10 times. After washing, the pellets are broken down into precipitate and dried at 50°C for 2hrs and CuO nanopowder was obtained.

### Characterization of CuO nanoparticles

### UV-vis spectroscopic studies

The absorption efficiency and the photon energy relationship are analyzed by UV-Vis spectroscopy based on their band gap energies of the tested nanoparticles.[22]

### Fourier Transform Infrared (FTIR) Spectroscopy

Perkin Elmer Spectrometer (JASCO V-650) is used to analyze the absorption spectrum of the tested nanoparticles with the ranges of 400 to 4000/cm by using potassium bromide pellets. By using FP-8200 spectrofluorometer, the photoluminescence capacity of the chemically synthesised CuO nanoparticles are analyzed.[22]

### X-ray diffraction (XRD) studies

CuO nanoparticles diffraction patterns are analysed by using powder XRD with the high scanning patterns of 10 to 80° with 2θ values.[22]

### Field emission scanning electron microscope (FESEM) and EDAX

After sonication, one drop of CuO NPs was kept on glass slide and air dried. Samples are coated with Pt and kept in the JSM6480LV SEM machine with accelerating microscope of voltage ranges between 10 to 20 kV. Energy Dispersive X-ray spectroscopy was performed for the compositional analysis on the CuO nanoparticles.[22]

### Experimental Organism

The earthworm, *Eudrilus eugeniae*[23] is an epigeic exotic species collected from Periyar University, Vallam (Tanjore dt., Tamil Nadu). For acclimatization, the worm are transferred to the lab. After 5 weeks acclimatization, the toxicity studies are initiated.[24] Adult earthworms are separated into groups ($n$=20) in separate containers with 10kg of soil.

### Lethal toxicity analysis

For the determination the acute toxicity of the CuO NPs, acclimatized adult earthworms are used.[25] Different concentrations of CuO NPs (200, 400, 600, 800, 1000, 1200 and 1400ppm) are prepared in 100ml of deionized water and mixed with 500gm of soil from the testing vessel. Twenty prewashed and ventilated mature earthworms are then introduced into each testing vessels and placed in an incubation chamber at 70 to 90% relative humidity, 23±1°C (12:12hrs). All the containers are covered with pieces of mesh cloth to prevent earthworms from escaping. During experimental period, mortality of the earthworm on each day/group are observed. After 14 days, mortality count of the earthworms are conducted by washing away the soil, and toxicity is calculated with the usage of regression analysis.

## RESULTS

### UV–vis absorption and FTIR spectroscopy

Absorption spectrum of CuO NPs can be detected commonly from the in the wavelength ranges of 300–800 nm. The UV-Vis absorption spectrum shown in Figure 1 and it reveals that the absorption threshold edge was found to be 366 nm. These result represented

the stabilization of copper with increased concentration of electrons.

FTIR spectroscopy results along with XRD patterns proved the purity of the synthesised CuO nanoparticles by chemical method. The peaks observed at 3422cm$^{-1}$ and 1610cm$^{-1}$ (Figure 1 and Table 1) were represented the stretching and bending vibrations of O-H groups respectively.

During nanoparticles synthesis, the optical absorption edge slightly shifted towards longer wavelength are due to the annealing mechanism enhanced upto 6hrs, mainly due to the increased grain size. The peaks observed at 2814cm$^{-1}$ and 1365cm$^{-1}$ were assigned as C-H stretching vibration and physisorbed H2O molecules on the surface of nanostructured CuO NPs. The absorption peaks in the range of 400 - 850cm$^{-1}$ were assigned to Cu-O and Cu-O-Cu lattice vibration. In our study, the peaks at 769 and 497cm$^{-1}$ denoted the stretching vibration of Cu-O bond in the monoclinic CuO.

## XRD and FESEM (EDAX)

Figure 2 showed the XRD patterns of the synthesised CuO nanoparticles at different time intervals. Different peaks were observed at (2θ) = 32.48° (111), 35.50° (002), 38.73°(111), 48.71° (-202), 53.62° (020), 58.26° (202), 61.53° (-113), 66.12° (022) and 68.04° (220) corresponds to different planes of monoclinic phase

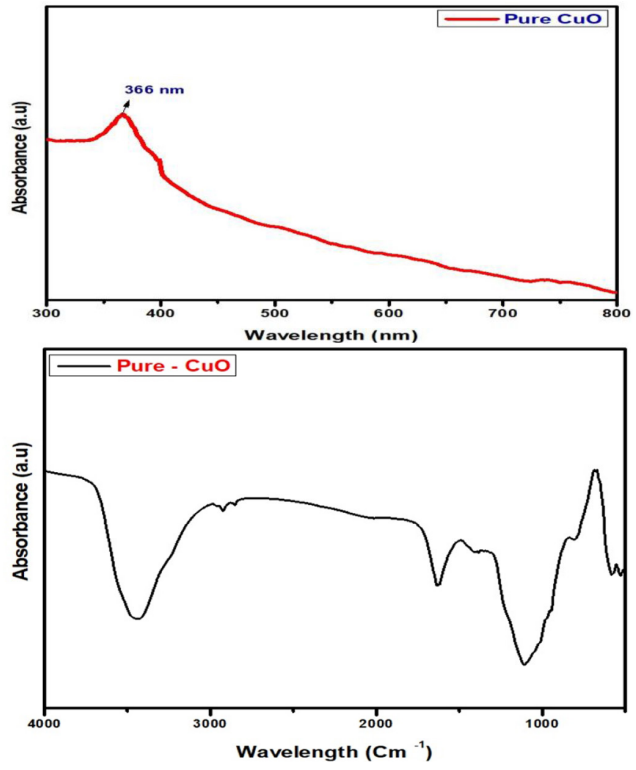


**Figure 1: UV and FTIR spectrum of synthesised CuO nanoparticles.**

**Table 1: FTIR peaks assignments of chemically synthesised CuO NPs.**

| | | |
|---|---|---|
| 1 | 3422 | O-H stretching |
| 2 | 2814 | C-H symmetric stretching |
| 3 | 1610 | H-OH bonding |
| 4 | 1365 | Physisorbed $H_2O$ stretching |
| 5 | 769 | Cu-O stretching |
| 6 | 497 | CuO stretching |

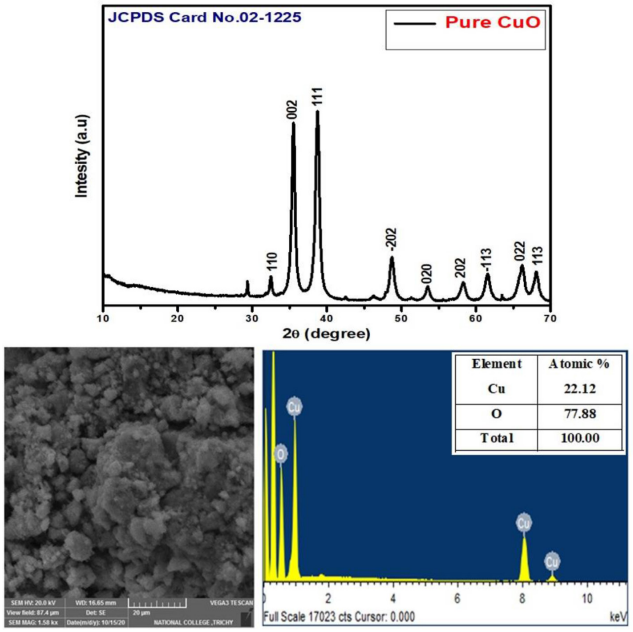


**Figure 2: XRD, FESEM and EDAX analysis of chemically synthesised CuO NPs.**

of CuO (with S.G. C2/c; and lattice constants (JCPDS No. 02-1225). It is clear that the major peaks located at 2θ = 35.43° and 38.49° are the characteristics peaks for the pure monoclinic phase of CuO NPs. The sharp and narrow diffraction peaks indicate that the material has good crystallinity and no other impurities were detected. The average grain size was found to be around 24 nm.

The chemically compositional analyses of the chemically synthesized CuO NPs were analysed by using EDAX (Figure 2). From the EDAX analysis, the chemical composition of Cu & O in chemically synthesized CuO nanoparticles are found to be 77.88% of oxygen, 22.12% of Cu respectively.

## Acute toxicity studies

The acute toxicity of the chemically synthesized CuO NPs on *E. eugeniae* earthworm was studied for 14 days. Based on the mortality results at the end of the experiment (Table 2, Figure 3), regression (Y=5.31X+9.67) results

**Table 2: CuO NPs mortality percentage of *Eudrilus euginea* (*n*=20).**

| Groups | Mortality (%) |
|---|---|
| Control | 10.5 |
| 300mg/kg | 34.8 |
| 600mg/kg | 36.7 |
| 900mg/kg | 40.9 |
| 1200mg/kg | 54.6 |

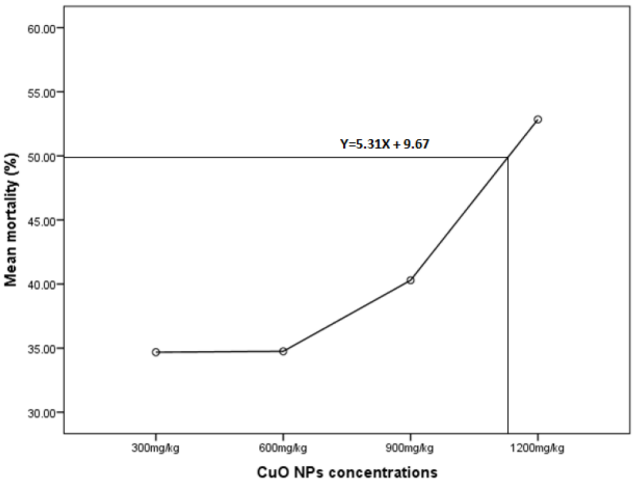


**Figure 3: Acute toxicity of CuO NPs on Eudrilus eugeniae.**

for the toxicity of CuO nanoparticles were observed in between the 1100 to 1200ppm group.

## DISCUSSION

Copperoxide nanoparticles UV analysis revealed the identification of peaks at 336nm confirmed the presence of Cu nanoparticles in the tested compound (Murendeni *et al.*)[26] Aftab *et al.*[27] also reported the chemically synthesized CuO NPs UV results evidenced at 370nm. Based on the literature comparison, the absorbance peaks observed in our CuO NPs (366nm) are similar with the ranges as 336 and 370nm.

Mwaanga *et al.*[28] reported similar results with stretching and bending vibrations groups. Marcano *et al.*[29] reported the metallic nanoparticles functional groups by FTIR characterization studies. Our results are evidenced by Zou *et al.*[30] reported about the FTIR analysis of chemically synthesised CuO nanoparticles whereas stretching and vibration groups are identified. Goswami *et al.*[32] and Karthik *et al.*[33] are studied about the crystal lattice plane of the CuO NPs and also their optical properties. The shape of the chemically synthesized CuO NPs are analyzed by FESEM image whereas Zou *et al.*,[30] Karthik *et al.*[33] and Erdogan and Gullu[34] also evidenced the presence of spherical shaped CuO NPs structures. Our results indicated the mortality percentage of the earthworm due to the exposure of the metallic copper oxide nanoparticles. Similar to our method, the direct exposure of Cu NPs cause serious effects on earthworms are reported.[14,15] Chemically synthesized Cu nanoparticles showed adverse effects on *Eisenia fetida* earthworm at >65ppm concentrations.[35] Pavani *et al.*[36] also reported the CuO NPs toxic effects on *Eisenia fetida* by both direct contact and soil contact methods whereas there is a significant relationship between the dose-related mortality. Mwaanga *et al.*[28] also evidenced the acute toxicity of the CuO NPs on *E. fetida* ranging between 100-4000ppm. Swart *et al.*[14,24] also studied the various impact of the metallic nanoparticles on the microbiome and earthworm.

Our results summary is metallic CuO NPs are synthesized by precipitate method. The nanoparticles composition, functional groups, optical properties and shape are studied with the help of various characterization methods. The acute toxicity of the CuO NPs are evaluated based on the soil emerging method for short period. The deleterious effects of CuO NPs on the *E. euginea* by short term studies confirmed the lethality of the CuO NPs and moreover, increased doses of the selected nanoparticles increased the toxicity in the adult *E. euginea* earthworm.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**CuO:** Copper oxide; **ppm: Parts** per million; **UV:** Ultra violet; **FTIR:** Fourier transform infra red; **FESEM:** Field emission scanning electron microscopy.

## SUMMARY AND CONCLUSION

Chemically synthesized CuO NPs characterization studies revealed the occurrence of spherical formed nanoparticles with 24nm size. EDAX results also

evidenced the presence of copper and oxygen molecules and the FTIR peaks stretching and vibrations also proved the presence of copper molecules. Acute toxicity (14days) of CuO NPs on *E. eugeniae* showed the mortality of earthworm and their $LC_{50}$ found between 1100-1200ppm. Our study concluded that the entry of CuO NPs through soil medium in low concentrations becomes toxic to the *E. eugeniae* earthworms. Our study proved that the synthesized metallic nanoparticles emergence into the soil ecosystem showed bioagumentation into the major invertebrate earthworm

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