



Vermicomposting and biomagnification of copper oxide (CuO) nanoparticles in *Eudrilus eugeniae*

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Abstract

Earthworms play a critical role in soil ecosystems and are widely used as bioindicators to assess terrestrial pollution. With the increasing release of metallic nanoparticles into the environment, their potential toxicity to soil organisms has become an important ecological concern. The present study investigates the impact of copper oxide nanoparticles (CuO NPs) at concentrations of 120 ppm, 150 ppm, and 180 ppm on the vermicomposting efficiency and biomagnification in *Eudrilus eugeniae*. Physico-chemical parameters of the resulting vermicompost, including pH, temperature, EC, TDS, organic carbon, macronutrients, and micronutrients, were analyzed after 60 days. Results showed a significant decrease in compost pH, nutrient content, and earthworm biomass in CuO NP-treated groups compared with controls, indicating reduced bioconversion ability. Micronutrients such as Ca, Mg, Mn, and Cu also declined significantly, suggesting inhibited metabolic activity and nanoparticle bioaccumulation within the earthworms. Overall, the study demonstrates that CuO nanoparticles adversely affect vermicomposting performance and nutrient quality, highlighting their ecological risks to soil health and organic waste management systems.

Keywords: *Eudrilus eugeniae*, copper oxide nanoparticles, vermicomposting, earthworm ecotoxicology, biomagnification, nanoparticle toxicity

Introduction

Earthworms are tiny invertebrates with soft, cylindrical bodies that are essential to the upkeep of soil ecosystems. It is a significant soil organism that has been benefiting humanity for millions of years. Utilizing earthworms to address a range of environmental issues, from trash management to soil enhancement, is a more recent development in the field of biotechnology known as "Vermiculture Technology." They invariably act as a "soil conditioner," enhancing the chemical, biological and physical characteristics of the soil as well as its nutritional content for robust plant development. Improved crop productivity is a result of worm activities such as soil fragmentation and aeration, organic matter decomposition and nutrient release, plant growth hormone secretion, nitrogen-fixing bacterial proliferation, and increased biological resistance in crop plants. Earthworms are vital for the stabilization of organic material and the preservation of soil structure [1, 2], as well as for the cycling of nutrients, primary production, and soil water management for the fertility of the soil [3, 4]. The *Eudrilus eugeniae* (night crawler) is a species of earthworm that is found worldwide [5,6]. It can grow at temperatures over 25°C, although it thrives around 30°C. It takes roughly 15 to 20 weeks to reach its maximum weight, length, and number of segments [7]. Its size can vary depending on the habitat and can range from roughly 10 cm to enormous specimens of over 12 cm [6]. Vermicomposting using this species results in biologically enriched compost with superior nutrient availability compared to conventional composts. Earthworm stocking density is a critical factor influencing vermicomposting efficiency.

Metal oxide nanoparticles such as copper oxide (CuO NPs) are increasingly released into the environment through industrial wastewater, sludge disposal, and agricultural

runoff. Although the toxic effects of nanoparticles on microorganisms and aquatic organisms are documented, there is limited understanding of their impact on soil invertebrates, particularly earthworms. Given their ecological importance and sensitivity to contaminants, earthworms serve as ideal bioindicators for assessing nanoparticle toxicity.

The present study investigates the effects of CuO nanoparticles on the vermicomposting capacity of *Eudrilus eugeniae*, focusing on biomagnification patterns and alterations in nutrient composition. This research aims to provide insight into how nanoparticle exposure disrupts soil biological processes and the ecological implications for sustainable waste management systems.

Materials and methods

The vermicomposting experiment was conducted using rectangular plastic tubs measuring 17 × 17 × 51 inches. Each container was thoroughly washed, sun-dried, and dusted with calcium powder prior to substrate preparation. Cow dung was used as the primary organic substrate. Copper oxide nanoparticles (CuO NPs) were incorporated into the substrate at concentrations of 120 ppm (Group I), 150 ppm (Group II), and 180 ppm (Group III). A nanoparticle-free control group was maintained.

The stocking density followed recommendations established for efficient vermicomposting, requiring a minimum of five earthworms per kilogram of organic material [8]. Fifty pre-weighed *Eudrilus eugeniae* individuals were introduced into each tub. The containers were kept indoors to avoid moisture loss, maintain temperature stability, and protect the worms from predators. Moisture levels were maintained between 50–55% through alternate-day sprinkling of water. The vermicomposting beds were aerated weekly by gentle turning to facilitate uniform decomposition without harming

the worms. Compost maturity was assessed based on granular structure, dark coloration, and an earthy odor. After 60 days, the vermicompost was collected by manual scraping of the upper layers.

Chemical analysis of the vermicompost included measurement of pH, temperature, electrical conductivity (EC), total dissolved solids (TDS), organic carbon, nitrogen, phosphorus, calcium, magnesium, sodium, potassium, chloride, sulphates, copper, and manganese. The analytical procedures followed standard soil-testing protocols as established by Santhi *et al.* [9]. Total nitrogen content was estimated using the Kjeldahl method.

All experimental data were expressed as mean ± standard deviation. Statistical significance between control and treatment groups was assessed using one-way analysis of variance (ANOVA), followed by least significant difference (LSD) testing. Statistical analyses were performed using SPSS software (Version 21).

Results

The physico-chemical parameters of vermicompost produced by control and CuO nanoparticle-treated groups showed significant differences after 60 days. pH levels decreased from 8.22 ± 0.34 in the control to 7.91 ± 0.53

(120 ppm), 6.88 ± 0.28 (150 ppm), and 6.21 ± 0.64 (180 ppm), indicating increasing acidity with higher nanoparticle concentrations. Temperature also declined significantly from 32.1 ± 0.9°C in the control to 30.2 ± 0.7°C, 29.8 ± 0.3°C, and 27.4 ± 0.3°C in Groups I–III, respectively, reflecting reduced microbial activity and slower decomposition. Electrical conductivity increased from 3.03 ± 0.34 mS/cm (control) to 3.45 ± 0.62, 3.89 ± 0.58, and 4.22 ± 0.47 mS/cm across the treated groups. Total dissolved solids also rose sharply, from 2345.4 ± 42.6 ppm in the control to 2904.4 ± 32.6 ppm, 3124.8 ± 38.9 ppm, and 3896.3 ± 55.7 ppm in Groups I–III, indicating a higher concentration of dissolved ions in the treated substrates. The organic carbon and nitrogen levels were considered as the very essential parameters due to the availability of the nitrogen in the compost for the growth of the plants. Organic carbon content increased slightly at 120 ppm (22.6 ± 0.39%) but declined at 150 ppm (20.7 ± 0.48%) and 180 ppm (18.7 ± 0.41%), compared to the control value of 19.7 ± 0.12%. Nitrogen content decreased progressively with nanoparticle exposure, from 2.1 ± 0.09% (control) to 1.8 ± 0.87%, 1.2 ± 0.11%, and 1.01 ± 0.06% at increasing concentrations.

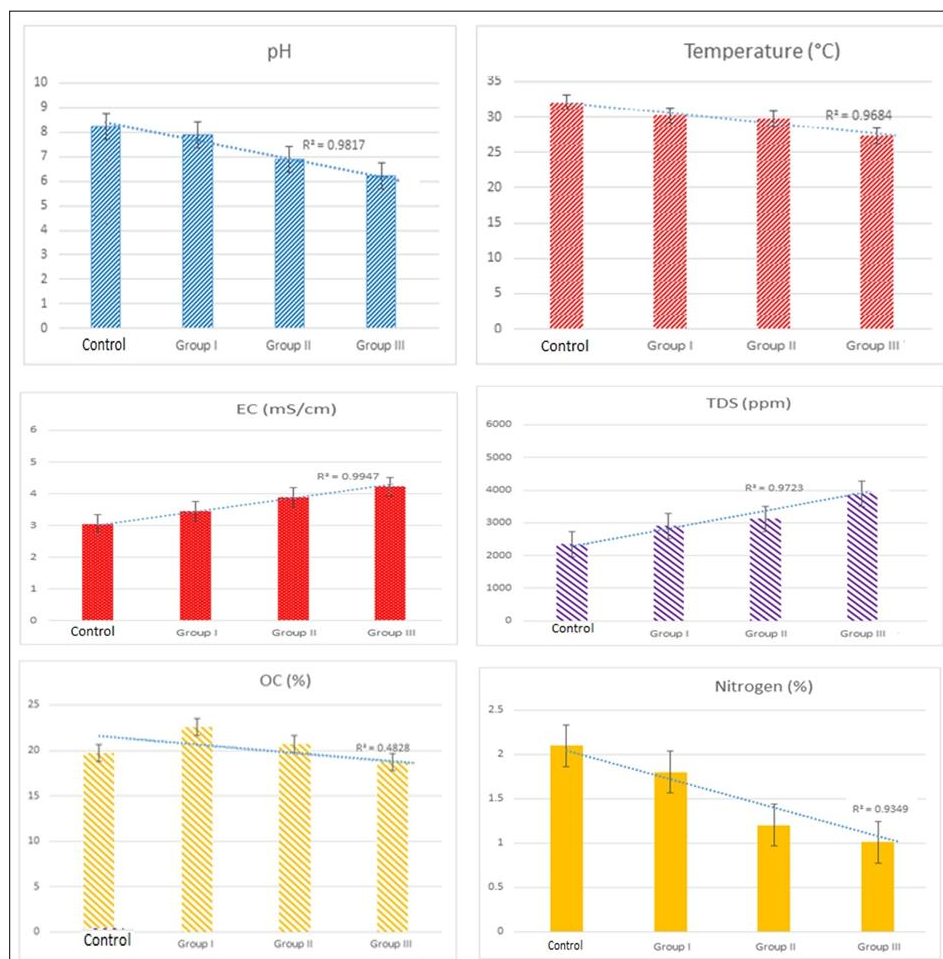


Fig 1: PH, temperature, Electrical conductivity, Total dissolved solids and the levels of Organic Carbon, Nitrogen in copper oxide nanoparticles treated *Eudrilus eugeniae* earthworms produced vermicompost

Macronutrients such as phosphorus, sodium, and potassium declined significantly in treated groups. Phosphorus decreased from 1.88 ± 0.11% (control) to 1.21 ± 0.16%, 1.08 ± 0.22%, and 1.02 ± 0.23%; sodium declined from 0.60

± 0.11% to 0.44 ± 0.13%, 0.36 ± 0.09%, and 0.30 ± 0.16%; potassium decreased from 1.01 ± 0.48% to 0.96 ± 0.31%, 0.72 ± 0.30%, and 0.69 ± 0.38%.

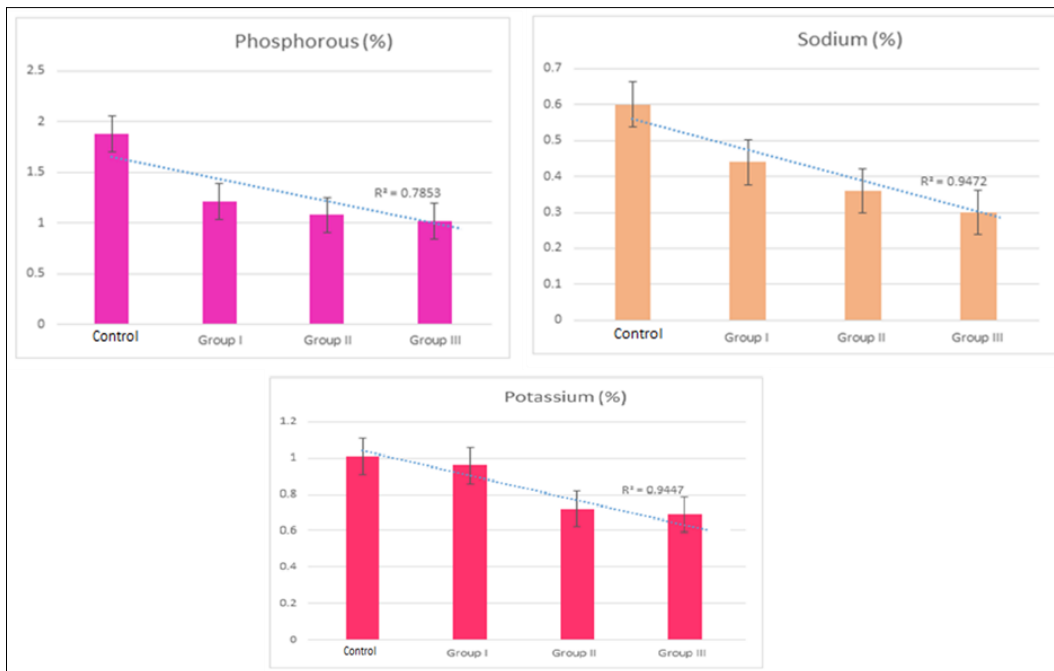


Fig 2: Levels of Macronutrients (Phosphorous, Sodium, Potassium) in copper oxide nanoparticles treated *Eudrilus eugeniae* earthworms produced vermicompost

Micronutrients also showed significant reductions. Chloride dropped from $1.78 \pm 0.11\%$ to as low as $1.30 \pm 0.21\%$ in Group III. Magnesium decreased from $1.20 \pm 0.18\%$ to $0.91 \pm 0.11\%$; sulphates from 1.06 ± 0.47 ppm to 0.64 ± 0.50 ppm; copper from 3.20 ± 0.37 ppm to 2.78 ± 0.17 ppm; calcium from 19.2 ± 1.11 ppm to 14.39 ± 0.19 ppm; and manganese from 3.62 ± 0.16 ppm to 2.16 ± 0.10 ppm. The initial weight of the earthworms (Figure 4) in all the

groups was found as 126.34 ± 3.01 , 128.68 ± 2.99 , 127.84 ± 2.01 , 128.11 ± 1.99 gm. After 60 days, the availability of the nutrients increases the weight as 245.38 ± 1.28 gm in control group whereas the treated group showed significantly decreased ($F=917.8$; $P<0.01$) body weight as 178.23 ± 1.27 , 145.32 ± 1.38 and 139.27 ± 1.28 gm for group I – 120ppm, group II – 150ppm, and group III - 180ppm CuO nanoparticles respectively.

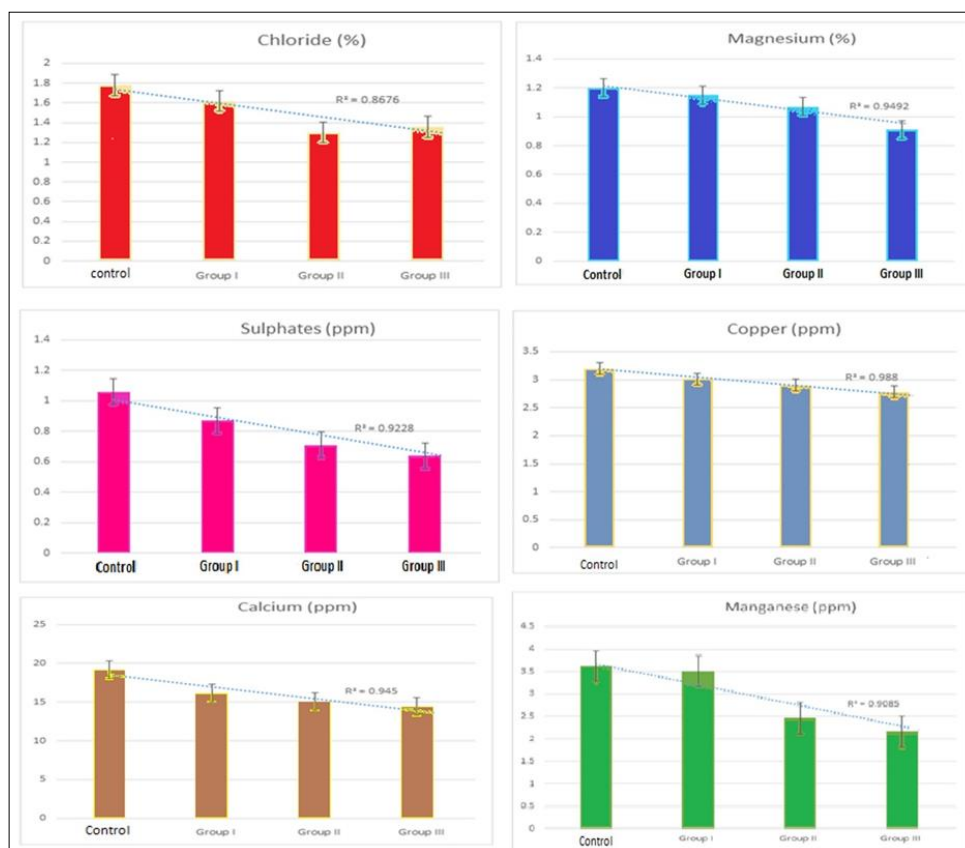


Fig 3: Changes in the Levels of Micronutrients in copper oxide nanoparticles treated *Eudrilus eugeniae* earthworms produced vermicompost

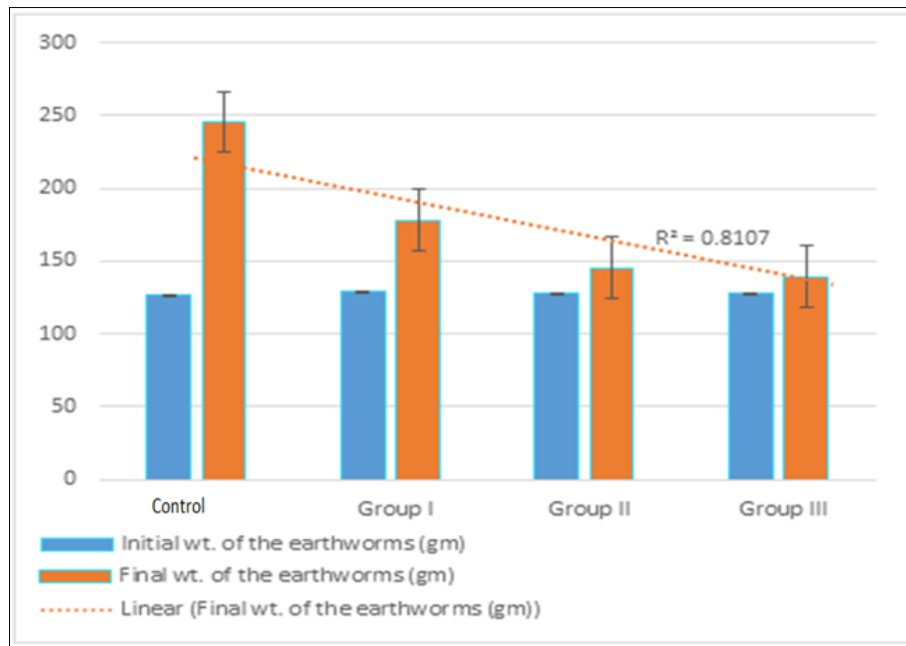


Fig 4: Effect of CuO nanoparticles on the body weight of *Eudrilus eugeniae*

Discussion

The findings of the present study demonstrate that copper oxide nanoparticles (CuO NPs) significantly alter the vermicomposting efficiency of *Eudrilus eugeniae*. Nanoparticles such as CuO, TiO₂, and ZnO have been shown to disrupt soil biological processes, and their increasing presence in treated sludge raises concerns about long-term environmental impacts [10-12]. The reduced nutrient mineralization and significant decline in physico-chemical quality of the vermicompost observed in this study support previous findings where metal oxide nanoparticles adversely affected earthworm physiology and microbial activity [13]. The observed decrease in pH, nutrient availability, and organic matter decomposition aligns with the documented alterations in microbial community structure and humification processes caused by nanoparticle exposure [13]. This suggests that CuO NPs impede the microbial–earthworm synergy essential for efficient vermicomposting.

The efficiency of vermicomposting is highly dependent on factors such as earthworm stocking density and substrate quality, as established by earlier studies [14-15]. The significant reduction in earthworm biomass in all treatment groups indicates a pronounced toxic effect of CuO NPs on growth, feeding activity, and metabolism. Such impacts are consistent with the well-established sensitivity of earthworms to soil contaminants and disturbances in their biological processes.

Earthworms produce mucus that maintains the pH between 6.5 and 7.5 to support the soil microbiota [16]. The progressive acidification seen in the treated groups suggests that CuO NPs may alter soil chemistry to an extent that disrupts both microbial activity and earthworm mucus mediated regulation of pH, which normally maintains biological equilibrium within the vermicomposting system. Earthworms are known to enhance nutrient enrichment in vermicompost through gut microbial interactions, enzymatic secretion, and nitrogenous excretions. However, the reduced levels of nitrogen, phosphorus, potassium, calcium, and magnesium in the treated groups suggest impaired mineralization and suppressed activity of beneficial

microbes. Previous studies have shown that earthworms facilitate a significant reduction in the carbon-to-nitrogen ratio during composting, contributing to substrate stabilization [17, 19]. In contrast, the reduced nutrient availability observed in this study indicates that CuO NPs interfere with these essential processes. Nanoparticles may also affect enzymatic and microbial mechanisms responsible for nutrient conversion, as documented in studies highlighting the influence of earthworm activity on the chemical transformation of organic waste [20, 21]. The reductions in calcium, manganese, and other micronutrients further support the hypothesis that nanoparticle exposure suppresses microbial biodegradation pathways and interferes with mineral cycling. Previous research indicates that earthworms significantly increase macronutrient and micronutrient content in vermicompost through their digestive processes and microbial interactions [18, 22, 24]. However, this study demonstrates that CuO nanoparticle exposure severely compromises this ability, resulting in nutrient-deficient vermicompost and diminished earthworm performance.

Overall, the findings reveal that CuO nanoparticles disrupt soil biological processes at multiple levels—microbial, chemical, and physiological—leading to reduced degradation efficiency, impaired earthworm health, and poor-quality vermicompost. Given the increasing environmental release of nanoparticles, these results highlight the need for stricter regulation, risk assessment, and further investigation into nanoparticle–soil–organism interactions to protect soil ecosystems and ensure sustainable composting practices.

Conclusion

The present study demonstrates that copper oxide nanoparticles (CuO NPs) significantly impair the vermicomposting efficiency of *Eudrilus eugeniae*. Exposure to increasing concentrations of CuO NPs resulted in reduced nutrient mineralization, decreased earthworm biomass, altered physico-chemical properties of vermicompost, and a decline in essential macro- and micronutrients. The findings also indicate bioaccumulation of copper within earthworm

tissues, suggesting that nanoparticles interfere with normal metabolic and digestive processes. These disruptions collectively lead to a reduction in vermicompost quality and highlight the ecological risks posed by nanoparticle contamination in soil ecosystems. The study emphasizes the need for monitoring nanoparticle release into the environment and recommends further research to understand the long-term effects of nanoparticle exposure on soil biota and organic waste recycling systems.

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