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Dose and Duration Driven Suppression of Exponential Population Growth of *Drawida willsi* Exposed to Copper Oxide Nanoparticles in Laboratory Conditions

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ABSTRACT

The increasing release of copper oxide nanoparticles (CuO NPs) into terrestrial ecosystems necessitates ecologically relevant risk assessment beyond conventional biomarker endpoints. The present study evaluated the dose and duration dependent effects of CuO NPs on the intrinsic rate of population increase (r) of *Drawida willsi*, an ecologically significant, r-selected tropical Earthworm species, under controlled artificial soil conditions. Population growth exhibited a clear concentration-dependent decline. While control groups maintained near-stable growth ($r \approx 0$ to -0.0065), exposure to ≥ 400 mg/kg CuO NPs resulted in pronounced negative r-values, reaching -0.0717 at 1000 mg/kg after 28 days. Two-way ANOVA revealed a highly significant effect of concentration ($F = 19.138$, $p<0.001$), whereas exposure duration (7-28 days) showed no significant independent influence ($p = 0.806$), indicating that toxicity was primarily dose driven. Tukey's post-hoc analysis identified significant suppression beginning at 400 mg/kg and highly significant inhibition at ≥ 600 mg/kg. These findings demonstrate rapid and sustained impairment of exponential population growth in *D. willsi*, highlighting demographic endpoints as sensitive indicators of nanoparticle stress. This study bridges a critical gap by integrating population growth analysis in a non-model tropical species, providing novel insights for soil nanoparticle risk assessment and long-term ecological sustainability.

Keywords CuO nanoparticles, *Drawida willsi*, Population growth rate (r), Soil ecotoxicology, Concentration-dependent toxicity.

INTRODUCTION

The rapid expansion of nanotechnology has led to the widespread production and application of engineered nanoparticles (ENPs) in agriculture, medicine, electronics, and environmental remediation. Among

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these, copper oxide nanoparticles (CuO NPs) are extensively utilized due to their antimicrobial, catalytic, and fungicidal properties. Their increasing incorporation into agrochemicals, fertilizers, pesticides, and industrial products has inevitably enhanced their release into terrestrial ecosystems (Gao *et al.* 2024, Islam *et al.* 2025). Once introduced into soil, CuO NPs may persist, transform, dissolve into ionic copper, or interact with soil biota, thereby raising significant ecotoxicological concerns (Naz *et al.* 2019). Given that soil acts as the primary environmental sink for metallic nanoparticles, understanding their impact on soil organisms is essential for ecological risk assessment.

Earthworms constitute a dominant component of soil macrofauna and are widely recognized as ecosystem engineers because of their fundamental contributions to soil structure and functioning. They actively participate in organic matter decomposition (Blouin *et al.* 2013, Lavelle *et al.* 2022). Through their burrowing and casting activities, earthworms enhance soil aggregation, improving soil porosity, aeration, and water infiltration (Bottinelli *et al.* 2020, Six *et al.* 2004). Their gut-associated processes and cast deposition further promote nutrient mineralization, (Aira *et al*. 2022, van Groenigen *et al.* 2014). In addition, earthworms regulate soil microbial communities by selectively grazing on microorganisms, modifying microbial biomass and diversity, and stimulating microbial functional activity through mucus secretion and gut transit effects (Ferlian *et al.* 2018, Thakuria *et al.* 2009). Collectively, these ecosystem services position Earthworms as key biological drivers of soil fertility and ecosystem productivity.

Their intimate contact with soil particles, ingestion of contaminated substrates, and permeable integument make them highly vulnerable to nanoparticle exposure (Spurgeon *et al.* 2020, Velicogna *et al.* 2021). Consequently, Earthworms have been extensively employed as bioindicators in soil ecotoxicology. Recent studies have demonstrated that CuO NPs can induce oxidative stress, cellular damage, reproductive impairment, and mortality in several earthworm species such as *Eisenia fetida* and *Allolobophora caliginosa* (Bakr *et al.* 2023). Chronic exposure has also been shown to alter gut microbiota, immune responses, and bioaccumulation patterns (Swart *et al.* 2020), while soil properties can modulate nanoparticle bioavailability and toxicity (Fischer *et al.* 2021, Jośko *et al.* 2021).

Although nanoparticle ecotoxicology has largely relied on standard laboratory species such as *Eisenia fetida* (Velicogna *et al.* 2021, Jośko *et al.* 2021), comparatively little attention has been given to native tropical earthworms, particularly species of the genus *Drawida*, despite their ecological importance in Asian soils (Narayanan *et al.* 2023). This is a critical limitation because species-specific life-history traits, burrowing behavior, and physiological tolerance can substantially influence contaminant sensitivity and exposure dynamics (Spurgeon *et al.* 2020, Pelosi *et al.* 2021). *Drawida willsi*, an r-selected species with high reproductive potential and rapid population turnover, remains largely unexplored in nanoparticle toxicity studies. Most existing investigations emphasize individual-level endpoints such as survival, biomass change, oxidative stress, and histopathology (Bakr *et al.* 2023), whereas demographic parameters like intrinsic rate of increase (r-value), which integrate survival and reproduction into a single ecological metric, are rarely assessed (Spurgeon *et al.* 2020). Given that population growth rate directly reflects ecological fitness and long-term sustainability in r-strategists, its evaluation is essential for ecologically meaningful nanoparticle risk assessment.

Furthermore, while concentration-dependent toxicity of CuO nanoparticles (CuO NPs) has been consistently documented in soil invertebrates (Naz *et al.* 2019, Velicogna *et al.* 2021, Jośko *et al.* 2021), the relative contribution of exposure duration versus concentration in determining population-level outcomes remains insufficiently resolved (Spurgeon *et al.* 2020, Pelosi *et al.* 2021). Many nanoparticle studies primarily focus on short-term biochemical and cellular endpoints-such as oxidative stress biomarkers, enzyme activity, and genotoxicity-without directly linking these responses to demographic consequences. Consequently, a substantial knowledge gap persists regarding how CuO NP exposure alters exponential population growth dynamics in ecologically relevant, non-model earthworm species under controlled soil conditions, particularly in tropical taxa that are underrepresented in nanotoxicological research (Velicogna *et al.* 2021, Narayanan *et al.* 2023).

The present study addresses this gap by evaluating the dose- and duration-dependent effects of CuO nanoparticles on the intrinsic rate of population growth (r) in *Drawida willsi* under laboratory conditions using artificial soil. By integrating demographic analysis with robust statistical evaluation (two-way ANOVA and post-hoc comparisons), this work moves beyond traditional toxicity endpoints to quantify ecologically meaningful population responses. The findings aim to (i) determine whether CuO NP exposure significantly alters exponential population growth in an r-selected earthworm species ; (ii) assess whether concentration or exposure duration exerts a stronger influence on demographic outcomes ; and (iii) identify potential threshold concentrations associated with significant population suppression.

By focusing on a native tropical species and employing population growth rate as a sensitive ecological endpoint, this study contributes novel insight into terrestrial nanoparticle ecotoxicology. The results are expected to strengthen environmental risk assessment frameworks by incorporating demographic parameters that better predict long-term ecosystem consequences of nanoparticle contamination in soils.

MATERIALS AND METHODS

Earthworms (*Drawida willsi*) were collected from agroecosystem site near Ranchi University, campus located between 21°58' to 25° 19'N and 83° 20' to 88° 4' E during morning hours following Srivastava *et al.* (2003). Earthworms were hand sorted and adult worms (clitellate, ≥ 4 cm) were used for experiment. Worms were acclimatized to laboratory condition for a week prior to start of experiment. Preparation of artificial soil was done by mixing soil, saw dust (presoaked in water for 3 weeks) and cow dung (dried and powdered) in 1:1:1 ratio (w/w in dry condition). CuO nanoparticles (< 50 nm particle size) were purchased from Sigma Aldrich (product no.544868-25G).

Pots used for experiments contained 1kg of artificial soil. To each pot containing artificial soil powdered CuO NPs was mixed at different concentrations (200 mg, 400 mg, 600 mg, 800 mg and 1000 mg). The effect of nanoparticles on Earthworm population growth was investigated at different concentration and different periods of exposure (7 d, 14 d, 21 d, and 28 d). For proper ventilation and exchange of gases the lid was perforated. A control was set up having only 1 kg of artificial soil with no CuO nanoparticle. The growth rate of Earthworm population has been calculated following Lotka equation. As pointed by Lotka (1925) the growth rate of a population (r) is expressed by the formula :

$$\frac{dN}{dt} = rN$$

Where, N is the number of individuals at any given time (t), which may be expressed as

$$N_t = N_0 e^{rt}$$

Where, e = base of natural logarithms and the parameter r in the equation describes the population growth.

OBSERVATIONS

The intrinsic rate of population increase (r) of *Drawida willsi*, an r-selected Earthworm species characterized by rapid multiplication under favorable conditions, showed a consistent decline with increasing concentrations of CuO nanoparticles (CuO NPs) in artificial soil. In the control treatment, the r-value remained stable at 0.00 during the initial 7 and 14 days, followed by only marginally negative values at 21 days (–0.0016) and 28 days (–0.0065), indicating near-stable population maintenance in the absence of nanoparticle stress (Table 1).

Exposure to 200 mg/kg CuO NPs resulted in slight reductions in r beginning at 14 days (–0.0049),

Table 1. Population Growth (r value) of *Drawida willsi* at different CuO Np conc. and Durations.

	7 days	14 days	21 days	28 days
Control	0	0	-0.0016	-0.0065
200 mg/kg	0	-0.0049	-0.0086	-0.0079
400 mg/kg	0	-0.0254	-0.0194	-0.0165
600 mg/kg	-0.026	-0.0289	-0.0267	-0.0182
800 mg/kg	-0.0579	-0.0326	-0.0336	-0.0429
1000 mg/kg	-0.0652	-0.0408	-0.0485	-0.0717

Table 2. Two-way analysis of variance (ANOVA) evaluating the effects of CuO nanoparticle concentration and exposure duration on the rate of population increase (r) of *Drawida willsi* under controlled soil conditions.

Source of variation	SS	df	MS	F	P-value	F crit
Rows (Concentration)	0.009123	5	0.001825	19.13826	4.96E-06	2.901295
Columns (Days)	9.34E-05	3	3.11E-05	0.326438	0.806266	3.287382
Error	0.00143	15	9.53E-05			
Total	0.010647	23				

with further modest declines at 21 days (–0.0086) and 28 days (–0.0079), suggesting mild suppression of population growth. At 400 mg/kg, the decline became more pronounced, with r reaching –0.0254 at 14 days and remaining negative through 28 days (–0.0165). A similar but stronger inhibitory pattern was observed at 600 mg/kg, where r was already negative at 7 days (–0.026) and reached –0.0289 at 14 days, persisting at reduced levels up to 28 days (–0.0182).

Marked growth inhibition was evident at higher concentrations. At 800 mg/kg, r declined sharply to –0.0579 at 7 days and remained strongly negative throughout the experimental period (–0.0429 at 28 days). The highest concentration, 1000 mg/kg, produced the greatest suppression, with r decreasing to –0.0652 at 7 days and further declining to –0.0717 at 28 days. These data demonstrate a clear concentration-dependent shift from near-zero growth in the control to pronounced negative growth rates at elevated CuO NP levels.

Two-way ANOVA (Table 2) revealed that concentration had a highly significant effect on population growth (F = 19.13826, p = 4.96 × 10^{-6}), whereas exposure duration did not exert a statistically significant influence (F = 0.326438, p = 0.806266). The F-value for concentration exceeded the critical value (Fcrit = 2.901295), confirming that variation in r was primarily attributable to differences in CuO NP concentration rather than exposure time within the 28-day period.

Tukey's post-hoc analysis further clarified inter-treatment differences (Table 3). No significant difference was observed between the control and 200 mg/kg treatment (p = 1.000). However, significant differences emerged between the control and 400 mg/kg (mean difference = 0.02293, p = 0.024), and highly significant differences were recorded between the control and 600 mg/kg (mean difference = 0.0397, p < 0.001) as well as 800 mg/kg (mean difference = 0.0545, p < 0.001). Similar patterns were observed when comparing 200 mg/kg with higher concentrations, particularly 600 mg/kg and above (p < 0.001). In contrast, comparisons among the highest concentrations (600–1000 mg/kg) were not consistently significant, indicating that beyond a threshold level,

Table 3. Tukey's honestly significant difference (HSD) post hoc multiple comparison test showing pair wise differences among CuO nanoparticle concentration treatments on the rate of increase (r) of *Drawida willsi*. . * p < .05, ** p < .01, *** p < .001.

		Control	200 mg/kg	400 mg/kg	600 mg/kg	800 mg/kg	1000 mg/kg
Control	Mean difference	—	0.00	0.0133	0.02293*	0.0397***	0.0545***
	p-value	—	1.000	0.351	0.024	<.001	<.001
200 mg/kg	Mean difference		—	0.0133	0.02293*	0.0397***	0.0545***
	p-value		—	0.351	0.024	<.001	<.001
400 mg/kg	Mean difference			—	0.00962	0.0264**	0.0412***
	p-value			—	0.676	0.008	<.001
600 mg/kg	Mean difference				—	0.0168	0.0316**
	p-value				—	0.149	0.001
800 mg/kg	Mean difference					—	0.0148
	p-value					—	0.249
1000 mg/kg	Mean difference						—
	p-value						—

additional increases in concentration did not proportionally intensify the inhibitory response.

Overall, the results demonstrate that CuO NP exposure significantly suppresses the exponential population growth of *D. willsi* in a dose-dependent manner. While duration of exposure up to 28 days did not independently influence growth rates, increasing nanoparticle concentration produced progressively stronger negative r-values, reflecting impaired reproductive performance and/or increased mortality. The findings clearly indicate that CuO NPs exert substantial ecological stress on this r-selected soil annelid under controlled laboratory conditions.

DISCUSSION

In the present investigation, CuO nanoparticle (NP) exposure induced a concentration-dependent suppression of population growth in *Drawida willsi*, an r-selected Earthworm species known for high reproductive output and exponential population dynamics under optimal conditions. In control treatments, r-values remained near zero during the first 14 days and only slightly negative by 28 days (–0.0065), consistent with expected maintenance of population stability in uncontaminated artificial soil. However, with progressive increase in CuO NP concentration, r-values shifted distinctly into negative territory, indicating suppressed growth and potential population decline across all treated groups.

The pattern of dose-dependent inhibition observed here agrees with recent ecotoxicological research documenting the adverse biological effects of metallic NPs on soil invertebrates. A 2025 study by Baxla *et al.* (2025) found significant concentration-dependent declines in Earthworm survival, biomass, and reproduction at CuO NP exposures similar to those used here, with severe toxicity at ≥600 mg/kg and near complete mortality at 1000 mg/kg. This underscores the ecological significance of NP contamination on soil fauna (Baxla *et al.* 2025).

The results from two-way ANOVA provide strong statistical support for the biological trend: Exposure concentration exerted a highly significant effect on r-values ($F = 19.138$, $p = 4.96 \times 10^{-6}$), whereas exposure duration from 7 to 28 days did not significantly influence population growth independently ($F = 0.326$, $p = 0.806$). This implies that toxic impacts were primarily driven by the magnitude of CuO NP exposure rather than cumulative exposure time within the 28-day period, suggesting rapid onset of toxic stress that stabilizes rather than progressively intensifies with longer exposure. Similar findings regarding rapid initial toxic responses to metal NPs have been reported for other invertebrate models ; for instance, antioxidant and immune biomarkers were significantly altered within short exposures to CuO and Ag NPs in *Allolobophora caliginosa*, indicative of early stress activation even at moderate NP levels.

Furthermore, post-hoc comparisons demonstrated that differences between control and low exposure (200 mg/kg) were not statistically significant ($p = 1.000$), while contrasts with higher exposures (≥400 mg/kg) were significant and highly significant at ≥600 mg/kg ($p < 0.001$). Such thresholds of toxicity align with prior soil toxicity benchmarks for CuO particles and ionic copper, where sublethal effects on reproduction and growth in Earthworms were observed at similar or lower concentrations in both model and natural soils (Spurgeon *et al.* as cited in Tatsi *et al.* 2018).

Mechanistically, the inhibition of population growth likely reflects a combination of physiological stress pathways triggered by CuO NP exposure. Nanoparticles can induce oxidative stress through generation of reactive oxygen species (ROS), lipid peroxidation, and cellular dysfunction, as documented in coelomic cells of Earthworms exposed to CuO NPs where malondialdehyde and DNA damage increased with exposure. Such subcellular disruptions are known to compromise reproduction, energy allocation, and survival, all of which manifest at the population level as reduced r-values. Further, metal oxide NPs including CuO have been shown to disrupt physiological barriers and facilitate uptake and accumulation of co-contaminants, exacerbating toxic stress in soil organisms (Naz *et al.* 2019).

In addition to direct organismal effects, CuO NPs may indirectly influence *D. willsi* by altering soil chemistry and the microbial environment. For example, research has demonstrated that CuO and other biocidal nanoparticles can significantly shift soil bacterial communities (with implications for nutrient cycling) even when negative impacts on Earthworm gut microbiota are buffered, suggesting that community-level changes could compound organismal stress (Naz *et al.* 2019, Swart *et al.* 2020).

Given the central role of Earthworms in soil structure, organic matter decomposition, and nutrient cycling, the observed reductions in population growth at ≥400 mg/kg CuO NP exposure carry important ecological implications. Earthworm declines at sublethal nanoparticle concentrations may reduce soil fertility and disrupt plant–soil interactions, effects previously observed with other nanoparticles such as silver sulfide, which negate the positive effects of Earthworms on plant nutrient uptake (Wu *et al.* 2024).

Taking together, the data presented here extends current understanding of CuO NP ecotoxicity in terrestrial systems by quantitatively linking exposure concentration to measurable declines in an r-species' population growth. The patterns and statistical robustness of these effects underscore the need for environment-specific risk assessments of engineered nanomaterials, particularly considering their widespread use in agriculture and industry and the potential for persistent soil accumulation (Singh *et al.* 2024, Tortella *et al.* 2024).

REFERENCES

Aira, M., Pérez-Losada, M., Crandall, K. A., & Domínguez, J. (2022). Composition, Structure and Diversity of Soil Bacterial Communities before, during and after Transit through the Gut of the Earthworm *Aporrectodea caliginosa. Microorganisms*, 10 (5), 1025. https://doi.org/10.3390/microorganisms10051025

Bakr, Z., Abdel-Wahab, M., Thabet, A. A., Hamed, M., El-Aal, M. A., Saad, E., Faheem, M., & Sayed, A. E. H. (2023). Toxicity of silver, copper oxide, and polyethylene nanoparticles on the Earthworm *Allolobophora caliginosa* using multiple biomarkers. *Applied Soil Ecology*, Volume 181,104681. https://doi.org/10.1016/j.apsoil.2022.104681.

Baxla, N. S., Subarna, S., Mandal, S. K., Srivastava, R., Singh, S., & Sinha, M. P. (2025). Concentration-dependent effects of CuO nanoparticles on survival, biomass and reproduction in *Drawida willsi* (Michaelsen). *Uttar Pradesh Journal of Zoology*, 46 (11), 67—73.

Blouin, M., Hodson, M. E., Delgado, E. A., Baker, G., Brussaard, L., Butt, K. R., Dai, J., Dendooven, L., Peres, G., Tondoh, J. E., Cluzeau, D., & Brun, J. J. (2013). A review of Earthworm impact on soil function and ecosystem services. *European Journal of Soil Science*, 64, 161—182. https://doi.org/10.1111/ejss.12025

Bottinelli, N., Kaupenjohann, M., Marten, M., Jouquet, P., Soucemarianadin, L., Baudin, F., Tran, T. M., & Rumpel, C. (2020). Age matters: Fate of soil organic matter during ageing of Earthworm casts produced by the anecic Earthworm *Amynthas khami*. *Soil Biology and Biochemistry*, 148, 107906.

Ferlian, O., Eisenhauer, N., Aguirrebengoa, M., Camara, M., Ramirez-Rojas, I., Santos, F., Tanalgo, K., & Thakur, M. P. (2018) Invasive Earthworms erode soil biodiversity: A meta-analysis. *Journal of Animal Ecology*, 87, 162—172. https://doi.org/10.1111/1365-2656.12746

Fischer, J., Evlanova, A., Philippe, A., & Filser, J. (2021). Soil properties can evoke toxicity of copper oxide nanoparticles towards springtails at low concentrations. *Environmental Pollution*, 270, 116084. https://doi.org/10.1016/j.envpol.2020.116084

Gao, J., Zhu, Y., Zeng, L., Liu, X., Yang, Y., & Zhou, Y. (2024). Recent advances on environmental behavior of copper-based nanomaterials in soil–plant systems: A review. *Journal of Environmental Management*, 361, 121289. https://doi.org/10.1016/j.jenvman.2024.121289

Islam, A. K. M. N., Uddin, M. N., Ridwan, A., Neon, A. K., & Rab, M. F. (2025). Engineering nanoparticles (ENPs) in aquatic environments and soil-plant ecosystems: Transformation, toxicity, and environmental challenges. *Frontiers in Soil Science*, 5, 1705689. https://doi.org/10.3389/fsoil.2025.1705689

Jośko, I., Kusiak, M., & Oleszczuk, P. (2021). The chronic effects of CuO and ZnO nanoparticles on *Eisenia fetida* in relation to the bioavailability in aged soils. *Chemosphere*, 266, 128982. https://doi.org/10.1016/j.chemosphere.2020.128982

Lavelle, P., Mathieu, J., Spain, A., Brown, G., Fragoso, C., Lapied, E., De Aquino, A., Barois, I., Barrios, E., Barros, M. E., Bedano, J. C., Blanchart, E., Caulfield, M., Chagueza, Y., Dai-J., Decaëns, T., Dominguez, A., Dominguez, Y., Feijoo, A., Folgarait, P., Fonte, S. J., Gorosito, N., Huerta, E., Jimenez, J. J., Kelly, C., Loranger, G., Marchão, R., Marichal, R., Praxedes, C., Rodriguez, L., Rousseau, G., Rousseau, L., Ruiz, N., Sanabria, C., Suarez, J. C., Tondoh, J. E., Valença, A. D., Vanek, S. J., Vasquez, J., Velasquez, E., Webster, E., & Zhang, C. (2022). Soil macroinvertebrate communities: A world-wide assessment. *Global Ecology and Biogeography*, 31, 1261—1276. https://doi.org/10.1111/geb.13492

Naz, S., Gul, A., & Zia, M. (2019). Toxicity of copper oxide nanoparticles: A review study. *IET Nanobiotechnology*, 14 (1), 1—13.

Lotka, A. J. (1925). *Elements of Physical Biology*. Williams & Wilkins, Baltimore, 460.

Narayanan, S. P., Kurien, V. T., Anuja, R., Hasyagar, V., Thomas, A. P., Paliwal, R., & Julka, J. M. (2023). Earthworm (Clitellata, Megadrili) fauna of Kuttanad wetland, Southern part of Vembanad-Kol Ramsar site, India. *Opuscula Zoolog-*

ica Budapest, 54, 3—21.

Pelosi, C., Bertrand, C., Daniele, G., Coeurdassier, M., Benoit, P., Nélieu, S., Lafay, F., Bretagnolle, V., Gaba, S., Vulliet, E., & Fritsch, C. (2021). Residues of currently used pesticides in soils and Earthworms: *A silent threat* ? *Agriculture*, *Ecosystems* & *Environment*, 305 (2021), 107167. https://doi.org/10.1016/j.agee.2020.107167

Singh, B., Kumar, K., Kain, T., & Singh, R. D. (2024). *In vivo* and *In vitro* studies of Cu-based nanoparticle toxicity in invertebrate worms: A review. EQA – *International Journal of Environmental Quality*, 63, 12—25. DOI: https://doi.org/10.6092/issn.2281-4485/19632

Six, J., Bossuyt, H., Degryze, S., & Denef, K. (2004). A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research*, 79 (1), 7—31. https://doi.org/10.1016/j.still.2004.03.008.

Spurgeon, D., Lahive, E., Robinson, A., Short, S., & Kille, P. (2020). Species Sensitivity to Toxic Substances: Evolution, Ecology and Applications. *Frontiers in Environmental Science*, 8 : 588380. https://doi.org/10.3389/fenvs.2020.588380

Swart, E., Dvorak, J., Hernádi, S., Goodall, T., Kille, P., Spurgeon, D., Svendsen, C., & Prochazkova, P. (2020). The Effects of *In Vivo* Exposure to Copper Oxide Nanoparticles on the Gut Microbiome, Host Immunity, and Susceptibility to a Bacterial Infection in Earthworms. *Nanomaterials*, 10 (7), 1337. https://doi.org/10.3390/nano10071337

Srivastava, R., Kumar, M., Choudhary, A. K., & Sinha, M. P. (2003). Earthworm diversity in Jharkhand state. *Nature Environment and Pollution Technology*, 2 (3), 357—362.

Tatsi, K., Shaw, B. J., Hutchinson, T. H., & Handy, R. D. (2018). Copper accumulation and toxicity in Earthworms exposed to CuO nanomaterials: Effects of particle coating and soil ageing. *Ecotoxicology and Environmental Safety*, 2018, 166, 462—473. https://doi.org/10.1016/j.ecoenv.2018.09.054

Thakuria, D., Schmidt, O., Liliensiek, A. K., Egan, D., & Doohan, F. M. (2009). Field preservation and DNA extraction methods for intestinal microbial diversity analysis in Earthworms. *J Microbiol Methods*, 2009 Mar, 76 (3), 226—33. https://doi.org/10.1016/j.mimet.2008.10.015.

Tortella, G., Rubilar, O., Fincheira, P., Parada, J., Caixeta de – Oliveira, H., Benavides-Mendoza, A., Leiva, S., Fernán dez-Baldo, M., & Seabra, A. (2024). Copper nanoparticles as a potential emerging pollutant: Divergent effects in the agriculture, risk-benefit balance and integrated strategies for its use. *Emerging Contaminants*, 10 (4), 100352. https://doi.org/10.1016/j.emcon.2024.100352

van Groenigen, J. W., Lubbers, I. M., Vos, H. M. J., Brown, G. G., De Deyn, G. B., & van Groenigen, K. J. (2014). Earthworms increase plant production: A meta-analysis. *Scientific Reports*, 4, 6365. https://doi.org/10.1038/srep06365

Velicogna, J. R., Schwertfeger, D., Jesmer, A., Beer, C., Kuo, J., DeRosa, M. C., Scroggins, R., Smith, M., & Princz, J. (2021). Soil invertebrate toxicity and bioaccumulation of nano copper oxide and copper sulphate in soils, with and without biosolids amendment. *Ecotoxicology and Environmental Safety*, Volume 217, 112222. https://doi.org/10.1016/j.ecoenv.2021.112222

Wu, J., Xiong, L., Huang, X., Li, C., Li, F., & Wong, J. W. C. (2024). Silver sulfide nanoparticles eliminate the stimulative effects of Earthworms on nutrient uptake by soybeans in high organic matter soils. *Science of The Total Environment*, Volume 947, 2024, 174433. https://doi.org/10.1016/j.scitotenv.2024.174433.