**Survivability of the Earthworm Perionyx sansibaricus (Michaelsen) in Soil Contaminated with Aluminium Oxide Nanoparticles**

**Abstract**

The present study examines the impact of aluminium oxide (Al2O3) nanoparticles (NPs) on the survivability of earthworm *Perionyx sansibaricus* (Michaelsen). Laboratory experiments were conducted to investigate the survival of *P. sansibaricus* at five different concentrations of Al2O3 NPs (50 mg kg⁻¹, 100 mg kg⁻¹, 300 mg kg⁻¹, 650 mg kg⁻¹, and 1000 mg kg⁻¹) over varying treatment durations (7 days, 14 days, 21 days, 28 days, and 52 days). Our study found that the survival rate of earthworms was 100% in the control group and at the low concentration (50 mg kg⁻¹) treatment. However, mortality increased significantly as the concentration of Al2O3 NPs increased. The average survivability at the doses of 100, 300, 650, and 1000 mg kg⁻¹ of Al2O3 NPs was 88.8%, 76.2%, 64.8%, and 53.2%, respectively. Notably, there was no significant difference in earthworm mortality across the different treatment durations.

**Keywords:** Earthworm *Perionyx sansibaricus*, Aluminium Oxide Nanoparticles, Survivability.

**Introduction**

The increasing production of nanoparticles and their byproducts is growing rapidly (Valerio-Rodríguez et al., 2020), leading to the intentional or unintentional release of nanoparticles (NPs) into the environment (Alahdadi & Behboudi, 2014). This release causes undesirable effects on the environment. Therefore, soil may serve as a sink for a significant fraction of the manufactured NPs released into the environment (Valerio-Rodríguez et al., 2020). Recent research studies (Jalili et al., 2020, Gudkov et al., 2022, Dube & Okuthe, 2023) have shown that NPs can induce toxic effects on aquatic organisms, rodents, and soil microorganisms. Earthworms are among the most important groups of soil invertebrates globally, in terms of both biomass and activity (Rombke et al., 2005). Earthworms can comprise 80-96% of invertebrates in some soils (Didden et al., 1994). They are well known to improve soil fertility by enhancing the physical, chemical, and biological characteristics of soil (Lee, 1985). Earthworms alter soil properties in ways that benefit plant growth by improving soil structure for better aeration, water intake, and water transmission, and they are known to have various beneficial effects on soil physical properties (Kimmins, 1987; Haynes et al., 2003; Rombke et al., 2005; Sautter et al., 2006).

Most published ecotoxicological studies on earthworms have focused on heavy metals (Parihar et al., 2019; Jairajpuri et al., 1993) and similar reviews on the effects of insecticides and pesticides on soil invertebrates, either in the laboratory (Booth et al., 2000; Marina et al., 2010) or in the field (Panda et al., 2000). Moreover, life cycle of the earthworm studied by Monroy et al., 2007 in the species *Octodrilus complanatus.* However, a review of the literature reveals that no reports are available on the impact of NP contamination on earthworms in the Indian subcontinent, where more than 500 species of earthworms are found (Kale et al., 1993). In light of this paucity of knowledge, the present paper aims to investigate the influence of Al2O3 NPs on the survivability of earthworms in artificial soil under laboratory conditions.

**Material and Methods**

**Test Soil**

The artificial soil was prepared as described in the OECD Test Guideline 207 (1984). Soil pH was measured using a pH meter. Organic carbon was determined by the Walkley & Black method (1934), while soil nitrogen was analyzed using the Kjeldahl method (1974). Soil phosphorus and potassium were analyzed following the method prescribed by Misra (1973). Soil moisture content was determined by the oven-dry method (Joshi et al., 2010). Some physicochemical properties of the test soil are presented in Table 1.

**Test Chemical**

Al2O3 nanoparticles (NPs) with a size of less than 50 nm were purchased from Sigma-Aldrich (Product No. 702129-100G). The particle size was confirmed by scanning electron microscopy (SEM).

**Characterization of Nanoparticles**

Scanning electron microscope (SEM) images of Al2O3 NPs were obtained from Birla Institute of Technology (BIT) Mesra (Ranchi, Jharkhand, India) using ZEISS microscopy (Figure 1), with the SEM operated in brightfield mode at 200 KX.

**Test Organism**

For the experiment, adult earthworms *P. sansibaricus* were collected from the soil using the hand-sorting method (Julka, 1993). The earthworms were identified by Zoological Survey of India, HQ, Kolkata. They were washed and acclimatized to the test soil under laboratory conditions for three weeks. Mature earthworms with developed clitellum were selected for the laboratory test. The average weight of *P. sansibaricus* was 0.67 ± 0.215 g per earthworm.

**Experimental Design**

The laboratory experiments were conducted at the Department of Zoology, Ranchi University, Ranchi. One kilogram of artificial soil was placed in a plastic circular pot (17 cm × 17 cm × 11.5 cm) and artificially contaminated by adding five different concentrations of Al2O3: C-0 (control group without Al2O3 NPs), C-1 (50 mg kg⁻¹), C-2 (100 mg kg⁻¹), C-3 (300 mg kg⁻¹), C-4 (650 mg kg⁻¹), and C-5 (1000 mg kg⁻¹). Each Al2O3 NPs concentration represented one experimental treatment setup. Ten healthy, fully ciliated earthworms were selected, rinsed, weighed, and placed in each of the plastic pots. Each treatment setups were incubated for 7, 14, 21, 28, and 52 days under laboratory conditions at a temperature of 23 ± 2 °C. To get best result each experimental setups were replicate thrice. The soil moisture level in the pots was maintained throughout the study by periodically sprinkling an adequate quantity of tap water. No organic matter was added during the entire experimental period to avoid affecting soil properties (Wu et al., 2020).

The earthworms were regularly monitored in the different experimental pots by counting and measuring the surviving earthworms in each pot. Earthworms were considered alive if they responded to mechanical stimuli (Anshu et al., 2020). After the experimental period, the earthworms were removed from the pots, and the total number of earthworms was counted for each experimental treatment.

**Statistical Analysis**

All data were analyzed using MS Excel (2018). The data analysis was performed using one-way analysis of variance (ANOVA).

**Results**

**Test Soil Characteristic**

The physicochemical characteristics of the artificial soil are summarized in Table 1.

**Table 1**: Main Physico-chemical characteristics of artificial soil (Mean ± SD)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| pH | Moisture  Level (%) | Organic C  (%) | Nitrogen  (%) | C/N ratio | Phosphorous (%) | Potassium (%) |
| 6.33 ± 0.40 | 51.67 ± 3.51 | 5.89 ± 0.39 | 0.34 ± 0.05 | 17.31 ± 2.00 | 0.34 ± 0.16 | 1.34 ± 0.10 |

**Characteristic of Nanoparticles**

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Fig.1: Scanning electron microscopic image of Al2O3 NPs

**Survivability of Earthworms Exposed to Al2O3 NPs**

The effects of different concentrations of Al2O3 NPs on earthworm survival rates at 7, 14, 21, 28, and 52 days are shown in Fig. 2. Our study found that the survival rate of earthworms was 100% for the control group and low concentration (50 mg kg⁻¹), while mortality significantly increased with higher concentrations of Al2O3 NPs. The average survivability for doses of 100, 300, 650, and 1000 mg kg⁻¹ of Al2O3 NPs was 88.8%, 76.2%, 64.8%, and 53.2%, respectively. However, there was no significant difference in mortality rates among earthworms at different treatment durations.

A one-way ANOVA test revealed no significant difference in the survivability of earthworms at a concentration of 50 mg kg⁻¹ of Al2O3 NPs. However, significant differences (p<0.05) were observed in survivability among earthworms treated with doses of 100, 300, 650, and 1000 mg kg⁻¹. No significant differences in mortality were noted across the various treatment durations (7, 14, 21, 28, and 52 days).

The percentage survival of earthworms after different doses and durations is shown in Figure 2.

Fig. 2: Effect of five concentrations of Al2O3 nanoparticles (C-0, C-1, C-2, C-3, C-4, C-5) on the survival of the earthworm *P. sansibaricus*: after 7, 14, 21, 28 and 52 days of Al2O3 NP treatment.

**Discussion**

Assessing the toxic effects of Al2O3 nanoparticles on earthworms is challenging due to the limited information available on aluminium in soil and the absence of environmental soil standards for Al2O3 (Zhao et al., 2010). In this study, exposure of earthworms *P. sansibaricus* to increasing concentrations of Al2O3 nanoparticles, ranging from 50 mg kg-1 to 1000 mg kg-1, resulted in decreased survival rates. Similar results were reported by Zhang et al. (2012), who conducted laboratory experiments examining the mortality of the earthworm *Eisenia fetida* due to aluminium toxicity across seven different Al2O3 concentrations, ranging from 0 to 300 mg kg-1 over 28 days. No aluminium toxicity was observed in earthworms when the Al2O3 concentration was ≤ 50 mg kg-1. Toxicity was noted at concentrations greater than or equal to 100 mg kg-1. The survival rate of *E. fetida* was 100% within the first 7 days of treatment, but this rate declined over time. Additionally, Zhao et al. (2010) reached similar conclusions regarding the acute toxicity of aluminium, noting a mortality rate in earthworm *E. andrei* after exposure to doses of 347, 416, 500, 600, 720, 864, and 1036 mg Al/kg dry soil after 7 and 14 days of treatment. The decline in survival of earthworms exposed to five different concentrations of Al2O3 nanoparticles over 52 days in the present study may be attributed to sub-lethal stress. These findings contrast with those of Bilalis et al. (2013) and Coleman et al. (2010), who reported no earthworm mortality in soil contaminated with Al2O3 at doses of up to 3000 mg/kg dry soil and 10,000 mg/kg Al2O3 nanoparticles, respectively.

**Conclusion**

Laboratory experiments were conducted to examine the survivability of the earthworm *P. sansibaricus* in Al2O3 nanoparticle-contaminated soil on the basis of different concentrations and durations. The survival of earthworms was influenced by varying doses of nanoparticles and the duration of exposure. Further field and laboratory research is necessary to assess the ecological and environmental impacts of the use and release of nanoparticles.

**Credit author statement**

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**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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**Declaration of competing interest**

The author (s) declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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