**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 (Lee et al., 2010) 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).

Earthworm biomass is a suitable indicator for determining soil pH, soil moisture, and organic layer (Muys & Granval, 1997). Organisms interact and establish themselves in ecosystems through populations, which are assemblages of individuals belonging to a particular species. Earthworm population dynamics, in both spatial and temporal terms, are influenced by multiple abiotic and biotic factors (Bisht et al., 2003). Most early population studies involved digging up soil samples and sorting them by hand (Stokki, 1928; Edward & Loft, 1977).

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). 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 (48). 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). 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. Ten healthy, fully ciliated earthworms were selected, rinsed, weighed, and placed in different plastic pots. Each treatment was incubated for 7, 14, 21, 28, and 52 days under laboratory conditions at a temperature of 23 ± 2 °C. 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., 2018).

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. 2a, 2b, 2c, 2d, and 2e. 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 Figures 2a, 2b, 2c, 2d, and 2e.

**2(a) 2(b)**

**2(c) 2(d)**

**2 (e)**

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*: (a) after 7 days of Al2O3 NP treatment, (b) after 14 days, (c) after 21 days, (d) after 28 days, and (e) after 52 days.

**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. (2021), 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 at five different concentrations (ranging from 50 to 1000 mg kg-1) over periods of 7, 14, 21, 28, and 52 days. 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.

**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.

**References**

Alahdadi I, Behboudi F (2014) The Effects of CuO and ZnO Nanoparticles on Survival, Reproduction, Absorption, Overweight, and Accumulation in *Eisenia fetida* Earthworm Tissues in Two Substrates. Int. J. Environ. Res. 9(1):35-42. <http://doi.org/10.22059/IJER.2015.871>

Allen SE, Grimshaw HM, Parkinson JA., Quarmbly C. Chemical Analysis of ecological material. London: Blackwell Scientific Publication, 565.

Anshu, Singh D, Mahima, Shweta (2020). Individual and combined toxic effects of Herbicides on growth parameter and fecundity of *Eisenia fetida*. International Journal of Current Microbiology and Applied Sciences, 9 (12): 1997-2005. [http://doi.org//10.20546/ijcmas.2020.912.236](http://doi.org/10.20546/ijcmas.2020.912.236).

Bilalis D, Sidiras N, Vavoulidou E, Konstantas A (2009) Earthworm populations as affected by crop practices on clay loam soil in a Mediterranean climate. Acta Agric. Scand Sect. B-Plant Soil Sci 59:440-446. <http://doi.org/10.1080/09064710802342327>

Bilalis, D., Tzortzi, I., Vavoulidou, E., Karkanis, A., Emmanouel, N., Efthimiadou, A., Katsenios, N., Patsiali, S., & Dellaporta L. (2013). Effects of aluminium and moisture levels on aluminium bioaccumulation and protein content in the earthworm *Octodrilus complanatus*. J. Soil Sci.Plant Nutr.13 (4): 845-854. [http://dx.doi.org/10.4067/S0718-95162013005000067](http://dx.doi.org/10.4067/S0718-95162013005000067%20)

Booth HL, Heppelthwaite, Halloran O’(2000). Growth, Development and Fecundity of the earthworm *Aporrectodea caliginosa* after exposure to two organophosphates. New Zealand Plant Protection. DOI: [10.30843/nzpp.2000-53 3692](file:///C:\Users\DELL\Desktop\SK%20AND%20SKM%20POPULATION\SK\10.30843\nzpp.2000-53%203692)

Coleman GJ, Jhonson RD, Stanley KJ, Bednar JA, Weiss AC, Boyd, ER, Steevens AJ (2010) Assessing the fate and effects of nano aluminium oxide in the terrestrial earthworm, *Eisenia fetida*. Environ Toxicol Chem.29 (7): 1575-1580.<http://doi.org/10.1002/etc.196>

Curry, JP. (2004). Factors affecting the abundance of earthworms in soils. In: Earthworm ecology. CRC Press; 401-426.

Edward CA, Bohlen PJ. (1995). *Biology and Ecology of Earthworm*. 3rd edition. London: Springer; 426.

Haynes R J, Dominy CS, Graham MH (2003). Effects of Agricultural land use on soil organic matter status and the composition of earthworm communities in Kwazulu-Natal, South Africa. Agric. Ecosyst. Environ. 95: 453-464. [http://doi.org/10.1016/S0167-8809 (02)00223-2](http://doi.org/10.1016/S0167-8809%20(02)00223-2)

Jairajpuri, MS (1993). Earthworm and Vermiculture: An Introduction. Earthworm Resources and Vermiculture. Zoological Survey of India, 1-6.

Joshi N, Dabral M, Maikhuri K R. (2010). Density, Biomass and Species Richness of Earthworms in Agrosystems of Garhwal Himalaya India. Tropical Natural History, 10(2): 171-179.

Kale, RD. (1993). Know-how of earthworm the soil macrofauna. Earthworm Resources and Vermiculture, Zoological Survey of India, 43-49.

Kimmins, J. P. (1987). Forest Ecology: A Foundation for Sustainable Management. Ist Edn. The University of British Columbia, Columbia, pp. 596.

Kooch Y, Jalilvand H, Bahmanyar MA, Pormajidian MR (2008) Abundance, Biomass and Vertical Distribution of Earthworm in Ecosystem Units of Hornbeam Forest. J. Biol. Sci. 8 (6):1033-1038. DOI: [10.3923/jbs.2008.1033.1038](file:///C:\Users\DELL\Desktop\SK%20AND%20SKM%20POPULATION\SK\10.3923\jbs.2008.1033.1038)

Lapied E, Nahmani J, Rousseau GX (2009) Influence of texture and amendments on soil properties and earthworm communities. Appl. Soil Ecol.43:241-249. doi:[10.1016/j.apsoil.2009.08.004](http://dx.doi.org/10.1016/j.apsoil.2009.08.004).

Lee Woo-Mi, Kim WS, Kwak J, Nam S-H, Shin Y-J, An Y-J (2010). Research trends of Ecotoxicity of Nanoparticles in Soil Environment. Toxicol. Res. 26 (4): 253-259. Doi: [10.5487/TR.2010.26.4.253](https://dx.doi.org/10.5487%2FTR.2010.26.4.253)

Lowe CN, Butt KR (2005) Culture techniques for soil dwelling earthworms: A review. Pedobiologia; 49: 401-413[. https://doi.org/10.1016/j.pedobi.2005.04.005.](file:///C:\Users\DELL\Desktop\SK%20AND%20SKM%20POPULATION\SK\.%20https:\doi.org\10.1016\j.pedobi.2005.04.005)

Marina S, Coviella C, Momo F (2014). Glyphosate Sublethal Effects on the Population Dynamics of the Earthworm Eisenia fetida (Savigny, 1826). Water Air Soil Pollut 225:2207. DOI: [10.007/s11270-0014-2207-3](file:///C:\Users\DELL\Desktop\SK%20AND%20SKM%20POPULATION\SK\10.007\s11270-0014-2207-3)

Misra, R. (1973). Ecology work book. Oxford and IBH Publ.Co. New Delhi, 243.

Muys B, Granval P (1997). Earthworms as bio-indicators of forest site quality. Soil Biol. Biochem.29:323-328.<https://doi.org/10.1016/S0038-0717(96)00047-8>

Organization for Economic Cooperation and Development (OECD) (1984). Guidelines for Testing of Chemicals. In No. 207, Earthworm Acute Toxicity Test.DOI: <https://doi.org/10.1787/9789264070042-en>

Ozawa T, Risal C P, Yanagimoto R (2005) Increase in the nitrogen content of soil by the introduction of earthworms into soil. Soil Sci. Plant Nutr. 51:917-920. <https://doi.org/10.1111/j.1747-0765.2005.tb00128.x>

Parihar K, Kumar R, Sankhla S M, Shefali (2019). Impact of Heavy Metals on Survivability of earthworm. International MLR. 2 (3): 51-52.

Römbke J, Jänseh S, Diden W (2005) The use of Earthworm in Ecological Soil Classification and Assessment Concepts. Ecolotoxicol. Environ. Saf. 62 (2):249-265. <https://doi.org/10.1016/j.ecoenv.2005.03.027>

Sautter KD, Brown GG, James SW, Pasini A, Nunes DH, Benito NP. (2006) Present knowledge on earthworm biodiversity on the state of Parana, Brazil. Europ. J. Soil Biol.: 42:296-300.

Panda S. Sahu KS (2000). Assessment of recovery of population, biomass and reproduction of the earthworm *Drawida willsi* following the application of malathion under field condition. Biol Fertil Soils, 32: 82-88.

Valerio-Rodrἱguez MP, Trejo-Tèllez LI, Aguilar-González MA, Medina-Pèrez G, Zúñiga -Enrἱqez, Ortegón-Pèrez A, Fernández-Luqueño F (2020) Effects of ZnO, TiO2 or Fe2O3 Nanoparticles on the Body Mass, Reproduction and Survival of *Eisenia fetida*. Pol. J. Environ. Stud. 29 (3), 2383-2394. DOI: [10.15244/pjoes/100668](file:///C:\Users\DELL\Desktop\SK%20AND%20SKM%20POPULATION\SK\10.15244\pjoes\100668).

Walkley, A., & Black, I. A. (1934). Determination of organic carbon in soil. Soil Science, 37; 29-38.

Wu J, Zhang C, Xiao L, Motelica-Heino M, Ren Z, Deng , Dal J. (2020). Impacts of Earthworm species on soil acidification, Al fraction and base cation release in a subtropical soil from China. Environmental Science and Pollution Research, 27: 33446-33457.[http:/doi.org/10.1007/s//356-019-050558](file:///C:\Users\DELL\Desktop\SK%20AND%20SKM%20POPULATION\SK\10.1007\s\356-019-05005-8)

Zhang, J., Yu, J., Ouyang, Y., & Xu, H. (2012). Responses of earthworm to aluminium toxicity in latosol. Environ. Sci. Pollut. Res.20, 1135-1141 DOI: [10.1007/s11356-012-0969-y](https://doi.org/10.1007/s11356-012-0969-y)

Zhao L & Qiu J.-P. (2010). Aluminium Bioaccumulation in the Earthworm and Acute Toxicity to the Earthworm, 4th International Conference on Bioinformatics and Biomedical Engineering. 1-4, doi: [10.1109/ICBBE.2010.5515226.](C:\\Users\\DELL\\Desktop\\SK AND SKM POPULATION\\SK\\10.1109\\ICBBE.2010.5515226)