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Keywords: Bioaccumulation; DNA damage; ZnO-NPs; Biodistribution

Introduction

Zinc oxide nanoparticles (ZnO-NPs) is among the most commonly utilized group of nanomaterials and has a wide ranging application [1]. As a well-known phytocatalyst, ZnO has received much attention in the degradation and complete mineralization of environmental pollutants [2-4]. ZnO-NPs are used in industrial products including cosmetics (sun screens, foot care, ointments and over-the-counter tropical products), pigments and coatings (ultra violet [UV]) protection, fungicide in paints), mouth washes, electronic devices and catalysts [5]. ZnO-NPs have also been used as a dietary supplement in human and live stock because Zn can stimulate the immune system and act in an anti- in ammatory way [6,7]. Many in vitro studies have demonstrated that ZnO-NPs are toxic to mammalian cells and even more toxic than other nanoscale structures of metallic oxide [8-10]. Some studies have reported that ZnO and its NPs have strong absorption abilities for a series of organic compounds and heavy metals [8,11]. In combination with UV exposure, ZnO-NPs are known to generate reactive oxygen species (ROS) like hydroxyl radicals or hydrogen peroxide in aqueous solutions leading to e cient decomposition of organic compounds [12]. Brunner et al. [13] showed that a three-day exposure of human mesothelioma and rodent broblast cell to ZnO-NPs (19 nm) caused DNA and mitochondrial changes. In addition to increasing our understanding of NPs toxicity, it is necessary to adequately study the properties of ZnO-NPs and, therefore, there is an urgent need to understand their toxicity in organisms and the environment through the processes of absorption, biodistribution, metabolism, and excretion of nanomaterials in vivo with a view to ensure that their applications are safe and provide helpful information to develop nanomaterial safety standard.

Earlier a series of physiological e ects induced by ZnO-NPs have been observed in rainbow trout, Oncorhynchus mykiss [14], micro algae Pseudokir- chneriella subcapitata [15], crustacean Daphnia magna and ermo- cephalus platyurus and bacteria Vibrio scheri [16]. However, research on the toxicity of NPs are far from being complete, and the studies of potential adverse e ects and related mechanisms exerted by NPs in the soil ecosystem are still limited [16-19].

During the life cycle of these commercial products, NPs may be released from products through normal use and then wastewater streams into the environment and become a threat to ecosystems. A signi cant portion of NPs in waste water are expected to release into sewage sludge [20,21]. Depending on local practices, varying proportions of sewage sludge are disposed of in land lls, incinerated, or applied to agricultural lands as biosolids. Usually 60-80% of sewage sludge is applied to the land [22]. erefore, terrestrial eco-systems are

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Materials and Methods

Characterization of ZnO nanoparticles

35 nm, a er 28 days . However, there was little increase in percentage of mortality at exposure of 10 nm NPs at >5 mg/kg. Study suggests that there is no signi cant mortality with decrease in size of NPs at the exposure <5 mg/kg. At higher concentration >4 mg/kg the particle size in coelomic uid was observed larger than its original size,

1), it was found that E. fetida survived even at a high concentration (10 mg/kg). Commercially < 5.0 mg/kg NPs are used as nano fertilizer for release of Zn in soil ecosystem. us, the highest concentration was considered to be 10 mg/kg, which represents a worst case scenario.

EWs did not show signi cant mortality at exposure of 100, 50 and evidences that this process also occurs for reduction of ZnO into Zn metal by EWs.

Increase in Cellulolytic activity

e cellulolytic activity of EWs' gut increased with the decrease in size of NPs (Table 3). Although, no statistically signi cant di erences appeared in the activity of cellulase as compared to control for 100 and 50 nm sized ZnO-NPs upto exposure 0f < 7.5 mg/kg. In contrast at exposing of 35 and 10 nm it increases 38-41%. Signi cant increase were recorded at the exposure of 10 nm @ >3.0 mg/kg. Results suggesting increase in cellulolytic activity in earthworm gut a er the exposure of ZnO-NPs may be helpful in and agree with the bioconversion process of lignocellulolytic wastes [26].

Cellulose is the most abundant polymer in nature and constitutes a large pool of carbon for microorganisms, the main agent responsible for soil organic matter decomposition. EW in uences decomposition 2.

However, percentage of mortality increased at exposure of 10 nm NPs at >5 mg/kg and there is no signi cant mortality with decrease in size of NPs at the exposure <5 mg/kg. At higher concentration (>4 mg/kg) the particles size in coelomic uid was observed larger than their original size, which indicates aggregation of the nanomaterial in the coelomic cavity of earthworms. No statistically signi cant e ects of any of the treatment on earthworm body mass were observed. e cellulase activity in earthworm gut was increased with the decrease in size of NPs. No signi cant correlation was observed between SOD and lignin peroxidase with the NPs that was neither concentration dependent nor size dependent. Statistically signi cant DNA damage was also not recorded in genotoxicity measurements with exposure of NPs. e study has demonstrated that bioavailability of ZnO-NPs was very high throughout the earthworm cross sections in all exposures of NPs particularly at exposure of 10 nm sized ZnO-NPs. e aggregate (100-200 nm) of NPs were also recorded within the cytoplasm. ere was no clear primary particles size dependence for accumulation on a mass concentration basis, although on a particle number basis, many more micro-size particles were observed. e evidence pre(er)138r8(v)-3

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