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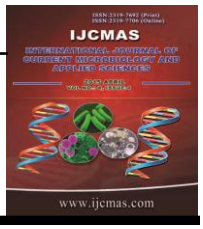

Original Research Article

Comparative study of Impact of ZnO-NPs on *Caenorhabditis elegans* and *Eisenia foetida*

Shweta Yadav*

Department of Zoology, School of Biological Sciences, Dr HS Gour Viswavidalaya, (A Central University), Sagar-470003, MP, India
**Corresponding author*

A B S T R A C T

In recent years use of nanomaterials have received much attention due to their unique properties.Apart from their being useful, nanoparticles can cause risks to environment from the beginning of the production to disposal. Therefore, the environmental risk assessment of nanoparticles is necessary. As regards earthworms are indicators of environmental pollution, the present study was take-up to compare impact of ZnO-NPs in earthworm *Eisenia foetida* and soil nematode *Caenorhabditis elegans***.** As nature has its ways of resolving imbalances in the environment and organisms is one of the best tools of nature to eliminate toxic pollutants. The biological process of eliminating pollutants (bioremediation) with activities of earthworms and associated gut micro-biome may translate to improve bioremediation process and to improve soil health. Study presents digital dynamics of trafficking and storage dynamics of ZnO-NPs in both soil organisms.

Keywords

ZnO-NPs, *Caenorhabditis elegans*, *Eisenia foetida*, bioremediation, bioaccumulation

Introduction

A rapid nano-technological advance suggests the wide application of metal oxide nanoparticles (MNPs) in various fields including medicines, catalytic activities, opto-electronic materials and environmental remediation practices. The growing use of these nanoparticles has led to their release into environment and soil is ultimate sink for released nanoparticles during their application. Therefore, is an essential to understand the impact of these nanoparticles on environment including soil organisms. MNPs belong to a family of nanomaterials that have been manufactured for industrial and household applications at a large scale. Among series of MNPs used in engineered nanotechnology, ZnO-NPs (zinc oxide nanoparticles) are widely used as nanosensors, UV-absorbers and catalysts. Yang *et al*. (2010) reported that ZnO-NPs have strong absorption abilities for various compounds including heavy metals. ZnO-NPs are widely used as ingredients in cosmetics as reflects UV light better than micro-particles. With their large commercial production, there may be unintended exposure to the environment. There is urgent need to understand *in vivo* impact of these

nanoparticles through the processes of absorption, bio-distribution, metabolism and excretion of nanomaterials.

As they may be released from various products through normal use and then enter into wastewater stream. A major portion of these nanoparticles may release into sewage sludge those are disposed of in landfills, incinerated or applied to agriculture lands. As soil system is an alternative sink for large portion of nanoparticles (Gottschalk *et al*., 2009). In our earlier studies (Gupta *et al*., 2014a) it was reported that coelomic cells of earthworms worked as nano- scavenger and prevent them to cause genotoxicity on earthworms activities. It was found that coelomic fluid of earthworms' discharges agglomerated/ hetero-aggregated forms of nanoparticles. The ingested organic matter helps to bind them firmly, so they worked as micro-particles in gut of earthworms. In continuation of earlier observations study aimed to compare absorption process of nanoparticles at same interval of time in both common soil organisms (*Caenorhabditis elegans* and *Eisenia foetida*) to understand their impact and in *vivo* absorption.

Materials and Methods

ZnO-NPs (50 nm, 35 nm) were purchased from Sigma-Aldrich. Particles were labelled as suggested by Tachikawa *et. al.* (2011) with fluorescent polymer.

The size of the particles was measured in 20-µl particle suspension from the test medium on 400 mesh carbon-coated copper grid and observed using a transmission electron microscope (40-100KV) at Sophisticated Analytical Instrumentation Facility of Electron Microscopy, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.

The wild-type *C. elegans* Bristol strain N2 was obtained from Caenorhabditis Genetic Centre (CGC), USA, and culture was maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* strain OP50 at 20°C, using the standard method (Brenner,1974). Young adult (3 days old) synchronized culture were used in all the experiments. Worms were incubated at 20°C for 24 h without a food source and were then subjected to the analysis (Van der Ploeg *et al*.,2011). Nematodes were exposed to three different-sized ZnO-NPs (35 and 50 nm). The test consisted 7 and 10 ml/l ZnO-NP concentrations. NPs were diluted in K-medium (32 mM KCl, 51 mM NaCl) following Williams and Dusenbery (1990) buffered in 140 mM sodium acetate (pH 6.0) to avoid aggregation. Each treatment was replicated for three times, and control (K-medium + buffer) was maintained for the entire test. After exposure of ZnO-NPs, fluorescence distribution images were observed by using fluorescence microscope equipped with a peltier cooled charge-coupled camera. Both differential interference contrast (DIC) and epi-fluorescence images were taken.

The research design to observe impact of ZnO-NPs in earthworms was developed following published OECD guidelines and Unrine *et al.* (2010). Twenty clitellate adult *Eisenia foetida* weighing 0.30 ± 0.12 g each in three replicate exposure chambers containing 1 kg dry mass of artificial soil medium were chosen for test experiment. The soil medium consisted of 70% quartz sand, 10% peat moss and 20% kaolin. The pH was adjusted with the addition of a small amount of crushed limestone. Two doses of 50, 35nm ZnO-NPs (10 mg/kg) were added to dry soil, mixed by homogenizer for 5 minutes and moisture content was maintained for 60%. Soil sub-samples were also taken at the beginning of the exposure

from each exposure chamber. After 24 hours of exposure, ten earthworm from each exposure chamber frozen at -80^{o}C for further analysis (not shown in present study). Three earthworm specimens were fixed in 2.5% glutaraldehyde and made into embedded sections following routine techniques for Transmission Electron Microscopy (TEM) characterization to examine the gut of worms.

Results and Discussion

The trafficking of accumulation of ZnO-NPs in *C. elegans* after 24 hrs of exposure of 50nm sized at 7ml/l and 10ml/l are shown in Fig 1 and 2. Findings revealed that exposure of 50nm ZnO-NPs at 7ml/l allowed to readily permeate into the body of *C.elegans* and accumulated in whole body including eggs. The ZnO-NPs efficiently translocated to distinct organs and tissues in *C. elegans* and additionally exhibits an ecotoxicity.

Since nematodes of the *Caenorhab-ditis* species are abundant inhabitants' soils and may thus participate in the food chain. The progeny production in *C. elegans* may be effected with permeated NPs. Notably, small sized particles (35 nm) rapidly permeated throughout the body even at low concentration (7ml/l) and shown high accumulation end points throughout gut (Fig 3 and 4). Several abnormalities on exposure of NPs including inflammation, absence of egg laying organs or defects in reproductive organs are shown in Fig 4. At present, it cannot be excluded that ZnO-NPs induced defective physiology of *C. elegans* due to rapid permeation and accumulation particles in gut as well reproductive organs.

The absorption of ZnO-NPs in *E.foetida at* different concentration in earthworm tissues are shown in transmission microphotographs Fig 5 and 6.The absorption results showed that earthworms uptake ZnO nanoparticles and accumulate in inter-coelomic spaces. The aggregation of nanoparticles not affects the mitochondrial activity. It may conclude the internalization of nanoparticles into their cell deviated from its real size and didn't generate oxidative stress.

Associated microbes of their gut helped them to bioaccumulate and bio-flocculate the nanoparticles (Gultom and Hu,2013; Reddy *et al*.,2012) and to deposit their homo and hetero-aggregates as well to reduce their toxic impact. The glycoproteins/ and or glycans of mucus released from their gut and associated microbes trapped nanoparticles. The glycoprotein molecules possess a vatiety of charges(positive N-bonds or negative O or P or S or C-O bonds) which allow particles to link together, resulting in a Zero point of charge as pH~2 for SiO_2; pH~6.5 for TiO_2; pH~8 for Fe_2O_3(Patwa *et al*.,2015) that may facilitate strong interactions between NP surfaces and glycoprotein. It is known that nanoparticles may adsorb on weak polyelectrolytes (Carnal *et al*., 2011). The charge distribution in the polymers,molecular weight (Mabire *et al*., 1984) and polymer coformation (Yan *et al*..,2014) may play an important role in formation and homo and hetro aggregates of nanoparticles.

Zheng *et al*.(2009) assayed the toxicity of ZnO-NPs in mice exposed *via* digestive tract and reported spleen and brain cells were normal, whereas other primary organs including heart, lung,liver and kidney were damaged. Same results were reported by Wang *et al*.(2012) and in our earlier studies (Gupta *et al*., 2014b) that pathological changes induced by ZnO-NPs were size and dose dependent.

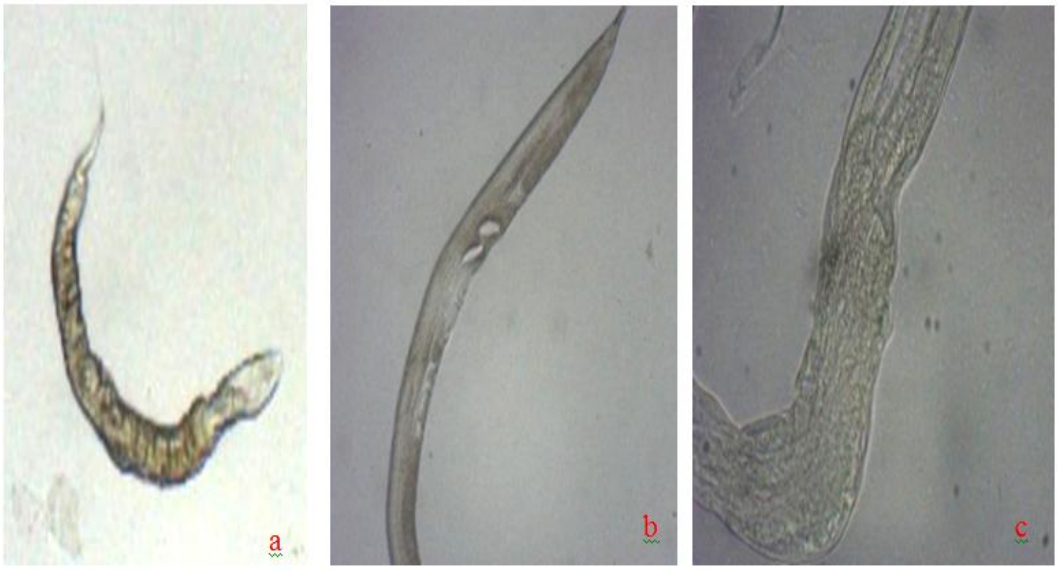

Fig.1 Accumulation of 50nm ZnO-NPs @ 7ml/l at 24 hrs: a, view of whole mount (10x); b, view of deposition of NPs around eggs (10x); c, deposition of NPs in gut (40x).

Fig.2 Accumulation of 50nm ZnO-NPs @ 10ml/l at 24 hrs: a, view of whole mount (10x); b, view of deposition of NPs in posterior region of gut (40x);c, view of deposition of NPs in intestine (40x).

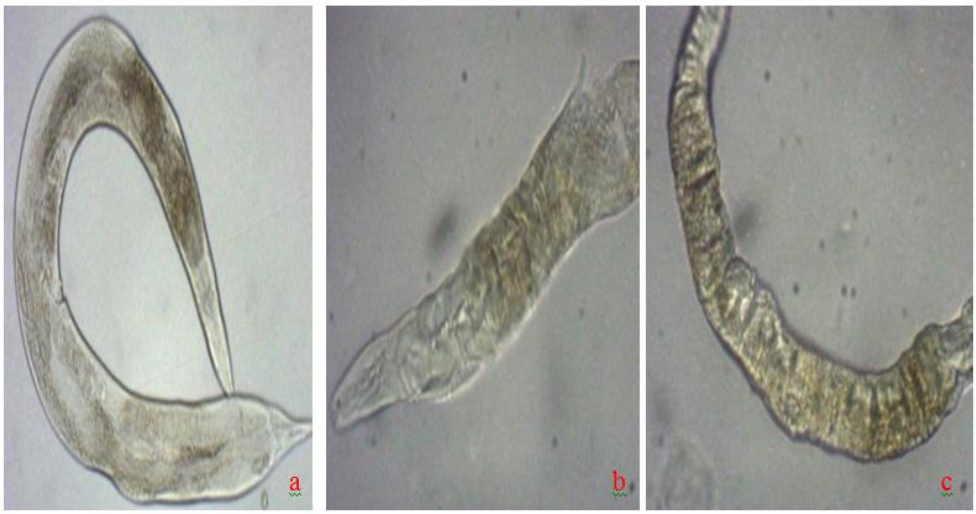

Fig.3 Accumulation of 35nm ZnO-NPs @ 7ml/l at 24 hrs: a, view of whole mount (10x); b, view of deposition of NPs in posterior region of gut (40x); c, view of deposition of NPs in intestine (40x).

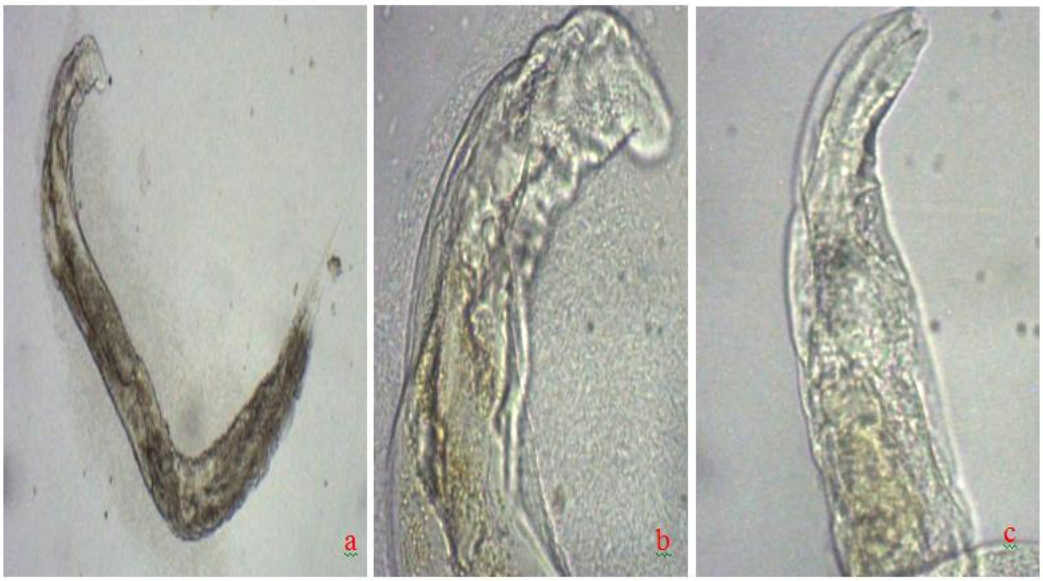

Fig.4 Accumulation of 35nm ZnO-NPs @ 10ml/l at 24 hrs: a, view of whole mount (10x); b, view of deposition of NPs in posterior region of gut (40x); c, view of deposition of NPs in pharynx (40x).

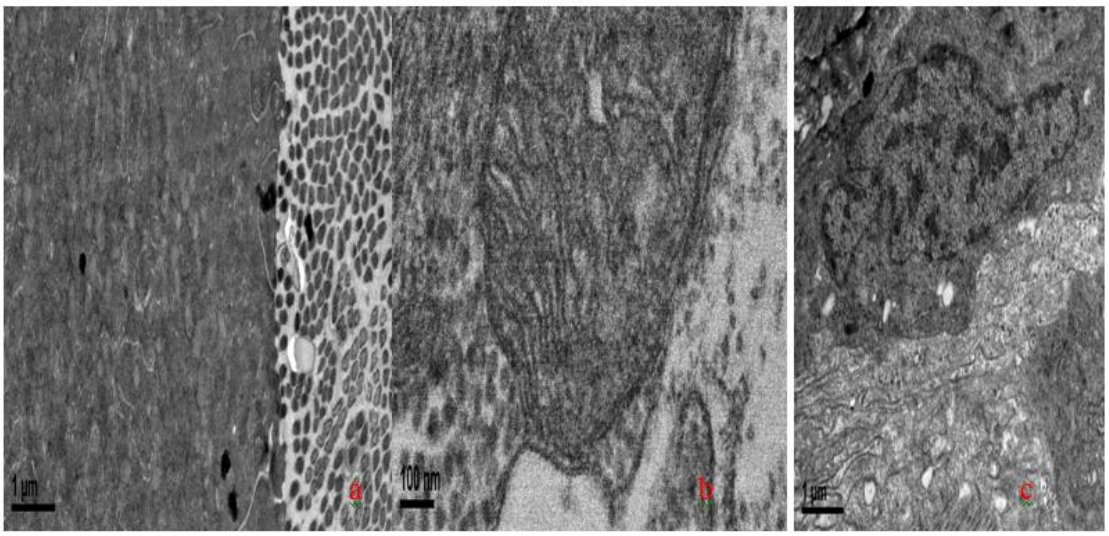

Fig.5 Transmission electron microphotograph of gut of *Eisenia foetida* at exposure of 50nm ZnO-NPs @ 10mg/kg at 24 hrs; a. view of permeation of exposure in gut; b, view of mitochondria cristae ; b, view of deposition of NPs in intercellular spaces.

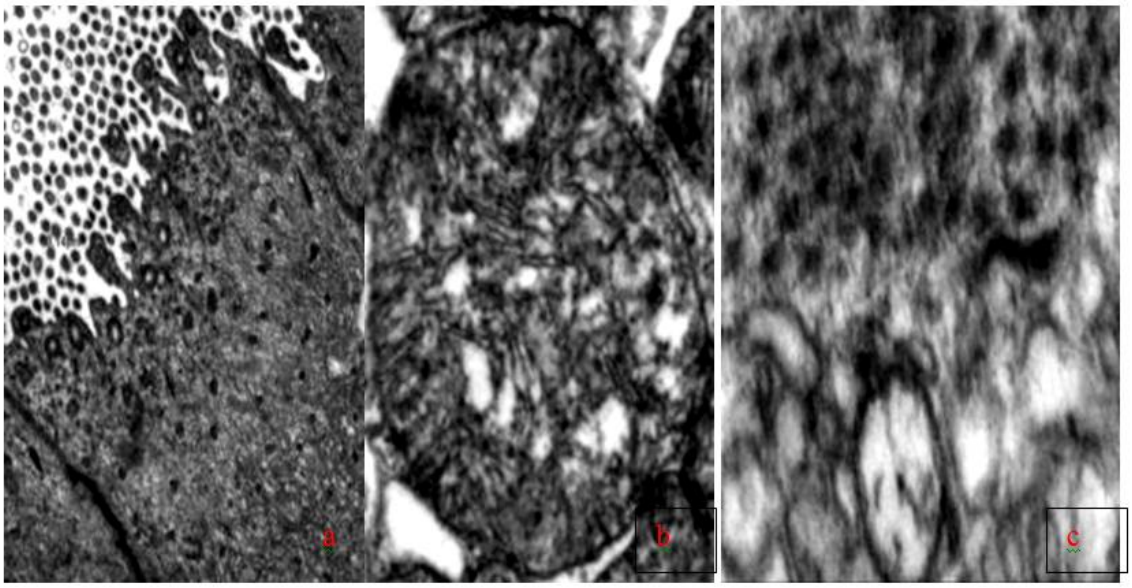

Fig.6 Transmission electron microphotograph of gut of *Eisenia foetida* at exposure of 35nm ZnO-NPs @ 10mg/kg at 24 hrs; a. view of permeation of exposure in gut; b, view of mitochondria cristae ; b, view of deposition of NPs in coelomic spaces

When mice were treated *via* the inter-tracheal tract, the histo-pathological observation revealed serious pulmonary inflammation, proliferation and alveolar wall thickening in the lung of all treated mice groups. Moreover all the changes were more serious in animals that received the higher doses. Horie *et al.* (2009) reported ZnO-NPs were strongly cytotoxic at low concentrations and exhibited strong protein

adsorption abilities which may be contribute towards their cytotoxicity. Bruner *et al*., (2006) found that almost human or rodent cells died following exposure of ZnO-NPs concentrations above 15ppm.Sharma *et al*., (2009) observed that ZnO-NPs toxicity was concentrations and time dependent. Study correspond to the finding of Wang *et al*., (2009) who reported ZnO-NPs can inhibit growth and reproductive activity of *Caenorhabditis elegans.*

It may concluded that earthworms can uptake and accumulate nanoparticles either through gut micro biomes or themselves. They are known to be potential bioaccumulator and therefore have been successfully demonstrated mitigating toxicity of industrial and municipal waste. They either "biotransform' or 'biodegrade' the chemical contaminants turning them harmless in their gut before entering of the nanoparticles in their tissues. Those entering in gut metabolized, immobilized and excreted or sequestered in tissues or vacuoles. Thus, earthworms might contribute in bioaccumulation and bio-flocculation of nanomaterials either with gut associated microbes or themselves and deposited them in the form of homo and hetero-aggregates in soil system. At the same time ZnO-NPs shown toxicity on growth and reproductive system of *C.elegans*.

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