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Research Article

ZnO NANOPARTICLES: CHARACTERIZATION AND ACUTE LETHAL STUDIES IN *EUDRILUS EUGENIAE*

Abbas, M., Meeramaideen M and Mohamed Shamsudin*

Department of Zoology, Jamal Mohamed College (Autonomous), Tiruchirappalli - 620020

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ABSTRACT

Engineered nanoparticles (NPs) are increasingly being used in a range of consumer products and entered into the environment. NP ZnO is one of the most widely used and potentially toxic NPs in soil organisms. The purpose of this study is to identify the particle size of the synthesized ZnO NPs by FTIR and UV-Vis spectroscopic which helps to investigate their effect on surface interaction with biological organisms. The synthesized ZnO NPs size is found as 47nm. *Eudrilus eugeniae* earthworm species is used for identify the median lethal concentration of ZnO NPs. The LC50 for ZnO NPs is observed between 1.80 to 2.00 gm/kg. Exposure of ZnO NPs to *Eudrilus eugeniae* cause toxicity while increased NPs concentration increases the impact.

INTRODUCTION

Nanoparticles (NPs) have received much attention not only due to their unique properties in optic, physical, chemical and biological processes, but also their potential effect on the ecosystem and human health (Moore, 2006; Wiesner *et al.*, 2006). Nowadays, the manufactured NPs are inevitably released into the environment during manufacturing, transport, use, and disposal operations either accidently or intentionally. So the fundamental understanding of their mode and range of toxicity is needed (Handy *et al.*, 2008; Lin *et al.*, 2010).

Metal oxide NPs are manufactured in large scale for both industrial and household purposes. Some authors reported increasing application of the NPs leads to environmental toxicity (Kahru *et al.*, 2008). Increased usage of nanoparticles leads to the purpose of developing the characterization of nanotoxicity of the particles to the biological organisms. Nanotoxicology helps to understand the size-specific behaviour and impact of nanoparticles on organisms and the environment (Ramesh, 2011).

Zinc oxide (ZnO) nanoparticles are mostly used as a UV light scattering, as an additive in cosmetics such as sunscreens, toothpastes and beauty products (Serpone *et al.*, 2007) and also widely used in rubber manufacturing process, for production of solar cells, LCDs, pigments (as a whitener), chemical fibers, electronics and textiles (Dastjerdi and Montazer 2010; Song *et al.*, 2010). It is an essential ingredient in almost all types of antifouling paints (IPPIC 2012). For the past two decades, the bulk ZnO has been increasingly replaced by ZnO NPs because of their enhanced antibacterial properties (Padmavathy and Vijayaraghavan 2008).

Soil represents a major recipient of nanoparticles entering the environment. Engineered nanoparticles may enter soil via wastewater treatment or the effluent from industrialization processes and many other anthropogenic activities which inhibit the organisms in the terrestrial ecosystems (Schlich *et al.*, 2012). Earthworms constitute 60-80% of the soil biomass (Rida, 1994). They are widely contributed in standard toxicity tests (Van-Straalen *et al.*, 1994) and appeared to be suitable biomonitoring organisms, particularly for their strong interaction and permanent direct contact with soil. The uptake, accumulation, and elimination properties of metals by earthworm are the major part of toxicological studies (Lee *et al.*, 2008).

The characterization of nanoparticles is used to identify their surface area and particle reactivity in solution, which are the important factors essential to identify the nanoparticles toxicity. Fourier transform infrared spectroscopy (FTIR) and UV-spectroscopy are the physical methods applied to study structure and properties of compounds. It is also performed to identify any functional groups adsorbing species onto the surface of the nanoparticles and to detect molecular structure.

Corresponding author:* **Mohamed Shamsudin
Department of Zoology, Jamal Mohamed College (Autonomous), Tiruchirappalli - 620020

MATERIALS AND METHODS

ZnO nanoparticles synthesis

For ZnO NPs synthesis, high purity chemicals such as Zinc (II) nitrate hexahydrate ($Zn(NO_3)_2$. $6H_2O$), Sodium hydroxide NaOH and Poly ethylene glycol (PEG) are used as the precursors without further purification. 0.1 M of Zinc Nitrate hexahydrate, 0.8 M of NaOH are dissolved 100 ml of distilled water (separately). NaOH solution is added drop wise into the Zinc Nitrate solution which yields white precipitate. The white precipitate is stirred in room temperature for 30min and in 60°C for 4 hours and kept the solution in the room temperature for 24 hours. The clear solution is collected and washed several times with double distilled water and ethanol. The white precipitate is dried 120°C and ZnO nanopowder obtained.

Characterization of ZnO Nanoparticles

For the synthesized particles, characterization studies are essential to know more about the particle size and nature of the particles which helps to study their biological interactions.

Fourier Transform Infrared (FTIR) Spectroscopy

The FT-IR spectrum is recorded in the range of 400-4000 cm^{-1} by using Perkin-Elmer spectrometer. The absorption spectrum of ZnO NPs is studied in the range between 190 and 1100 nm by JASCO V-650 spectrophotometer. Photoluminescence spectra are taken using spectrometer JASCO spectroflurometer FP-8200. The samples for FT-IR analysis are pelletized using KBr.

UV- Vis-NIR spectroscopic studies

The relation between the absorption coefficients α and the incident photon energy hυ can be written as

$$\alpha h\upsilon = A(h\upsilon - E_g)^n$$

where E_g is the optical band gap, A is the constant and the exponent n depends on the type of transition. The value of n = 1/2, 3/2, 2, or 3 depends on the nature of the electronic transition (1/2 for allowed direct transition, 2 for allowed in-direct transition, 3/2 and 3 for forbidden direct and forbidden indirect transition respectively).

Experimental Organism

The earthworm *Eudrilus eugeniae* species are collected from Panikkam Patty, Tiruchirappalli district, Tamil Nadu and transported to the Environmental Research laboratory Department of Zoology, Jamal Mohamed College (Tiruchirappalli) for acclimatization. The worms are acclimatized for 30 days before initiation of experiment. Experimental soil for worm rearing is prepared by mixing cow dung and degraded organic waste like dried leaves. Humidity of the soil is maintained by water spraying at regular intervals. The adult earthworms are collected and separated into groups. Groups are maintained in plastic tubs (45x30x15cm) contains 8kg of soil.

Acute Toxicity Test

The groups are exposed to different concentrations of synthesized ZnO nanoparticles as 0.5, 1, 2 and 3 gm/kg respectively. The parameters are maintained as per the standard norms. For every 12 hrs, the groups are observed for the dead earthworms. Mortality rate is observed and dead ones are removed immediately to avoid contamination of the soil.

Statistical Analysis

The toxicity results are interpreted by using SPSS software (version 17.0). The median lethal concentration of ZnO nanopaticles is studied by probit analysis and also with the Regression values.

RESULTS AND DISCUSSION

Characterization of the manufactured engineered nanoparticles is essential for the development of biological interacting measurements and also provides basic informations about their properties such as molecular size, shape and its measurements provides uncertainty estimation to ensure the reliability of the characterization of nanoparticles (Powers *et al.*, 2006; Motzkus 2013).

The FT-IR spectrum of the prepared ZnO is shown in Figure 1. The FT-IR measurements are performed for the samples using the KBr pallet method in the wave number range 400-4000 cm^{-1}. The broad absorption in the frequency band 3750-3000 cm^{-1} is assigned to O-H stretching from residual alcohols, water and Zn-OH (Senthilkumaar *et al.*, 2008).

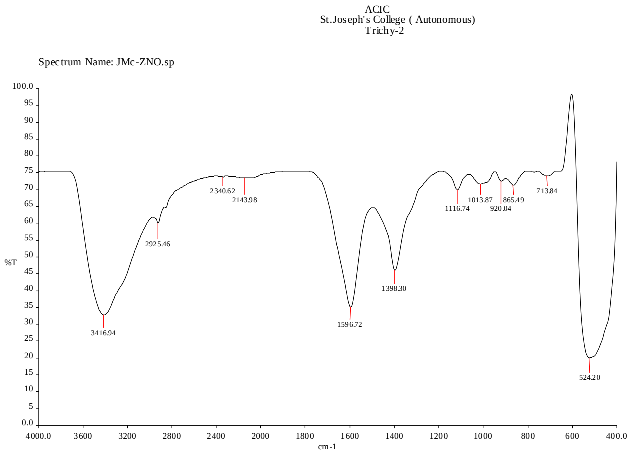

Figure 1 FTIR spectrum of ZnO nanoparticles

The absorption peak is observed at 3436 cm^{-1} for the ZnO NPs sample. The CO_2 peak is observed at 2340 cm^{-1} for ZnO samples. These CO_2 bands may arise due to some trapped CO_2 in air ambience (Oo *et al*., 2005) The H-OH bending vibration is around 1596 cm^{-1} for ZnO sample. The Zn-O stretching bands are observed at 524 cm^{-1} for the ZnO NPs sample. Figure 2 showed the absorbance of the ZnO NPs sample depended on several factors such as band gap, oxygen deficiency, surface roughness and impurity centers. The excitonic peaks are observed around 384 nm for the ZnO.

The acute toxicity of ZnO NPs to earthworm species *Eudrilus eugeniae* increased with particle concentration, demonstrating a dose dependency. The observations were done manually and recorded. By using probit analysis, the 50% of the earthworm mortality was observed between 1.80 to 2.00 gm/kg ZnO nanoparticles (Fig 3) in three replicates. Engineered metal nanoparticles ZnO, TiO_2, Ag and CeO toxicity will at least partly be due to the release of free metal ions (Auffan *et al.*, 2009), while effects may further be enhanced by the specific properties related to the small size and consequent high surface activity of the particles. So it is essential to study the characterization of the nanoparticles before commencing experiments.

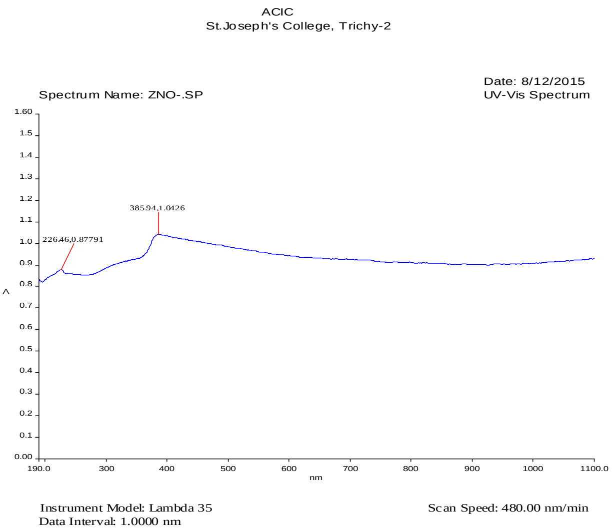

Figure 2 UV-Vis spectrum of ZnO nanoparticles

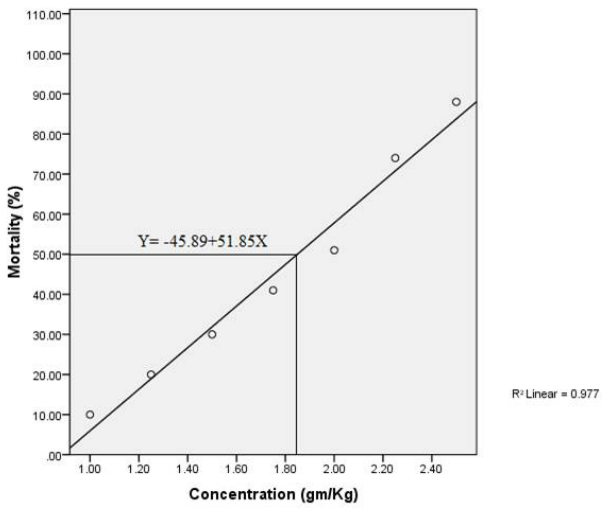

Figure 3 Median Lethal (LC50) toxicity of ZnO nanoparticles exposed to the earthworm *Eudrilus eugeniae*

Earthworm serves as "bioindicators" to understand the physicochemical characteristics of their habitat. They are common in a wide range of soil and may represent 60-80% of the total soil biomass and an ideal organism for use in soil toxicity evaluations (Bouché, 1992). Li *et al*. (2010) studied toxicity effects of ZnO and TiO_2 NPs on *Eisenia fetida* and have shown that toxicity and accumulation increase with increasing NPs concentrations. Hu *et al*. (2010) evaluated toxicity of 0.1, 0.5, 1.0 or 5.0 g/kg of TiO_2 and ZnO NPs to the earthworm *Eisenia fetida* exposed for 7 days resulted in various abnormalities.

CONCLUSIONS

FTIR and UV-Spectroscopic results helps to identify the characteristics of synthesized ZnO NPs and their nanoparticle size is 47nm. The acute toxicity of ZnO NPs to earthworm species *Eudrilus eugeniae* increased with particle concentration, the median lethal concentration is observed between 1.80 to 2.00 gm/kg. Based on the present study results, ZnO nanoparticles are found to be toxic to earthworm *Eudrilus eugeniae* and further elaborate studies such as morphological, histological and biochemical analysis are to be done.

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