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Biotoxicity of Metal Oxide Nanoparticles (ZnO) on Soil Biological Properties

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ABSTARCT

Nanotoxicity, an emerging concept, is receiving increasing attention with the fast development of nanotechnology. The present toxicity study was carried out to assess the toxicity potential of metal oxide nanoparticles (ZnO) on soil biological properties. The experiment result indicates the soil biological properties like microbial population (bacteria, fungi and actinomycetes) and enzyme activity (dehydrogenase, urease and phosphatase) were significantly reduced by the presence of higher concentration of zinc oxide metal nanoparticles in the soil.

Keywords Nanotoxicity, ZnO nanoparticle, Soil, Microbes, Enzymes.

INTRODUCTION

The development of nanotechnology and the manufacture of new organic and inorganic nanosized particles or Engineered Nanoparticles (ENPs) may result in the release of substantial amounts of these materials into the environment. This production includes engineered nanoparticles made for use in agriculture applications and environmental remediation. However, because of their widespread use in consumer products, it is expected that engineered nanoparticles will find their way into aquatic, terrestrial and atmospheric environment (Navarro *et al.* 2008). The fate and transport of nanosized materials, once they are released into the environment, has not yet been fully addressed, nor have the impacts of those materials on plant system and soil communities (Dionysiou 2004). The main characteristic of engineered nanoparticles is their size (<100 nm), which falls in the transitional zone between individual atoms or molecule and the corresponding bulk materials. This can modify the physico-chemical properties of the material as well as create the opportunity for increased uptake and interaction with biological systems. This combination effect can generate adverse biological effects in living cells that would not otherwise be possible with the same material in large form (Nel *et al.* 2006). Other properties of engineered nanoparticles, such as high specific surface area, abundant reactive sites on the surface as a consequence of a large fraction of atoms located on the exterior rather than in the interior of engineered nanoparticles, as well as their mobility, could potentially lead to unexpected health and environmental hazards (Maynard *et al.* 2006). Therefore, organisms and especially those that interact strongly with their immediate environments are expected to be affected as a result of their exposure to engineered

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Fig. 1. Pot culture experiment.

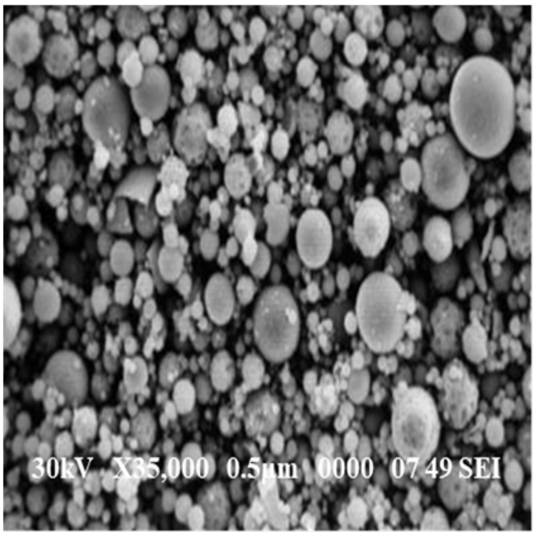

Fig. 2. Zinc oxide (ZnO) nanoparticles.

nanoparticles.

Nanoparticles are introduced into the soil as a result of a number of human activities, including deliberate releazes viz., soil and water remediation technologies, potential agricultural uses (eg. Fertilizer) and unintentional release viz., air, water and sewage applied to the lands (Shah and Belozerova 2009). Microorganisms play a very important role in maintaining the soil health, ecosystem functions and production. Many nanoparticles have already been reported to have anti-microbial properties and thus directly affect microorganisms. Microbial toxicity has been reported for titanium dioxide and fullerene nanoparticles (Lovern and Klaper 2006). Soni and Bondi (2004) elucidated the mechanisms of action of how silver nanoparticles damaged bacterial cells. They reported that silver nanoparticles damaged and pitted the cell wall of *Escherichia coli* and accumulated in the cell wall, leading to increased cell permeability and ultimately cell death. Nanoparticles of zinc oxide and magnesium oxide have been shown to be efficient in killing microorganisms and are used as preservatives in food. Iron and copper-based nanoparticles could be presumed to react with peroxides present in the environment generating free radicals. These radicals are known to be highly toxic to microorganisms (Sabila *et al.* 2006). To date, very little data exist on the toxicity of nanoparticle and limited toxicity studies was reported on the effect of nanoparticles on soil microbial community. Hence, the present study was carried out to assess the toxicity potential of ZnO nanoparticles on soil biological propertires.

Table 1. Effect of zinc oxide (ZnO) nanoparticles on soil microbial populations.

Treatments	Microbial population		
	Bacteria × 10^6 CFU g^{-1}	Fungi × 10^4 CFU g^{-1}	Actino-mycetes × 10^3 CFU g^{-1}
T_1	22.8	12.8	8.80
T_2	22.1	12.4	8.50
T_3	21.7	11.8	8.50
T_4	19.2	11.2	7.90
T_5	17.6	9.80	6.70
T_6	15.8	8.60	5.50
T_7	11.8	6.40	4.60
Mean	18.7	10.4	7.20
SEd	1.57	0.87	0.61
CD (0.05)	3.37	1.87	1.31

MATERIALS AND METHODS

A pot culture experiment was carried out with maize plant to assess the effect of zinc oxide nanoparticles soil biological community (Fig.1). The experiment consists of seven treatments with three replications. The treatments include T_1 - Control (without nanoparticles), T_2 - Nano ZnO - 100 mg kg^{-1} of soil, T_3 - Nano ZnO - 500 mg kg^{-1} of soil, T_4 - Nano ZnO - 1000 mg kg^{-1} of soil, T_5 - Nano ZnO - 2000 mg kg^{-1} of soil, T_6 - Nano ZnO - 5000 mg kg^{-1} of soil and T_7 - Nano ZnO - 10000 mg kg^{-1} of soil. The Completely Randomized Design (CRD) was followed for the experiment.

Collected soil was dried under shade, powdered with wooden mallet. Then the soil was passed through 2 mm sieve to remove pebbles, roots and debris. After processing, one kg of soil was transferred to each pot and required nutrients were added. Based on the treatments, nanoparticles were added to each pot and thoroughly mixed for 15 min. Two maize seeds were sown in pots of each replication. The plants were watered once in two days. The experiment was conducted for 35 days (up to vegetative stage) and the effects of ZnO nanoparticles on the soil biological community was observed and recorded. The soil samples were collected prior to the pot culture experiment and also collected at end of the experiment to assess the initial and final status of soil biological properties.

RESULTS AND DISCUSSION

The surface area of the ZnO nanoparticles was measured to be 15 to 25 m^2 g^{-1}. The molecular weight of the particle was 81.39. The typical SEM image (Fig. 2) shows ZnO nanoparticles are predominately spherical in shape with the size of 20 to 85 nm. The soil supported considerable amount of microbial population and enzyme activity. The initial microbial population of bacteria, fungi and actinomycetes were 23.9 x 10^6, 12.8 × 10^4 and 9.10 × 10^3 CFU g^{-1} of soil, respectively. The enzyme activity viz., dehydrogenase, urease and phosphatase were 5.81 µg of TPF g^{-1} of soil, 8.95 µg of NH_3 released g^{-1} of soil h^{-1} and 31.32 µg of PNPP g^{-1} of soil, respectively.

In this present study, the soil biological properties like microbial population (bacteria, fungi and actinomycetes) and enzyme activity (dehydrogenase, urease and phosphatase) were significantly reduced by the presence of higher concentration of zinc oxide nanoparticles in the soil. The population of bacteria, fungi and actinomycetes were significantly reduced under 10000 mg of nano ZnO kg^{-1} of soil followed by 5000 and 2000 mg of nano ZnO kg^{-1} of soil (Table 1). This might be due to the presence of higher concentrations of metal oxide nanoparticles in soil leads to produce more toxic effect on microbial populations. The results obtained are in line with the findings of Lovern and Klaper (2006), who reported that titanium dioxide and fullerene nanoparticles were significantly cause toxic effects on microorganisms. Soni and Bondi (2004) also analyzed the effect of silver nanoparticles on bacterial cells. They reported that the silver nanoparticles damaged and pitted the cell wall of *Escherichia coli* and accumulated in the cell wall, leading to increased cell permeability and ultimately cell death. Nanoparticles of zinc oxide and magnesium oxide have been shown to be efficient in killing microorganisms and most of the nanoparticles could be presumed to react with peroxides present in the environment generating free radicals. These radicals are known to be highly toxic to microorganisms (Sabila *et al.* 2006). The effects of nanoparticles on general soil processes and nutrient cycling were evaluated using three different enzyme (dehydrogenase, urease and phosphatase) activities. These enzyme activities are excellent indicator of soil microbial function, which are key component in nutrient cy-

Table 2. Effect of zinc oxide (ZnO) nanoparticles on soil enzyme activities.

Treatments	Enzyme activity: Dehydrogenase (µg of TPF g^{-1} of soil)	Urease (µg of NH_3 released g^{-1} of $soil^{-1}$)	Phosphatase (µg of PNPP g^{-1} of soil)
T_1	5.56	8.70	30.58
T_2	5.24	8.32	29.95
T_3	5.02	8.07	28.26
T_4	4.85	7.86	26.83
T_5	4.00	6.68	23.38
T_6	3.48	5.73	22.11
T_7	2.36	4.05	17.53
Mean	4.36	7.06	25.52
SEd	0.37	0.59	2.11
CD (0.05)	0.79	1.27	4.54

cling (Roldan *et al.* 2005). Phosphatase is important enzyme for catalysis of organic phosphatase esters into inorganic phosphate, which is important in phosphorus mineralization and processing (Amador *et al.* 1997). Dehydrogenase activity was assessed as a general indicator of oxidative capacity of soil microorganisms (Williams and Drewes 2006). Urease was assessed because of its role in the hydrolysis of urea, the major organic form of nitrogen, and its implications in nitrogen uptake and cycling (Sukul 2006). The phosphatase, dehydrogenase and urease activity were significantly influenced by the ZnO nanoparticles (Table 2). Among the concentrations tested, the phosphatase, dehydrogenase and urease activity were significantly reduced at the concentration of 10000 mg of nano ZnO kg^{-1} of soil followed by 5000 and 2000 mg of nano ZnO kg^{-1} of soil. A similar phenomenon was observed by Tong *et al.* (2007) and he reported that the enzymes like dehydrogenase, urease, β-glucosidase and phosphotase activity, structure and function of soil microbial community and microbial process were affected due to the presence of fullerene nanoparticles at the concentration of 1000 mg kg^{-1} of soil.

CONCLUSION

The toxicity study reveals that the possible adverse effect of metal oxide nanoparticles to direct exposure on soil biological community, which underscore the need for ecological responsible disposal of waste or sludge containing metal oxide nanoparticles and also calls for further research on the potential impacts of manufactured nanoparticles on agriculture and environmental systems.

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