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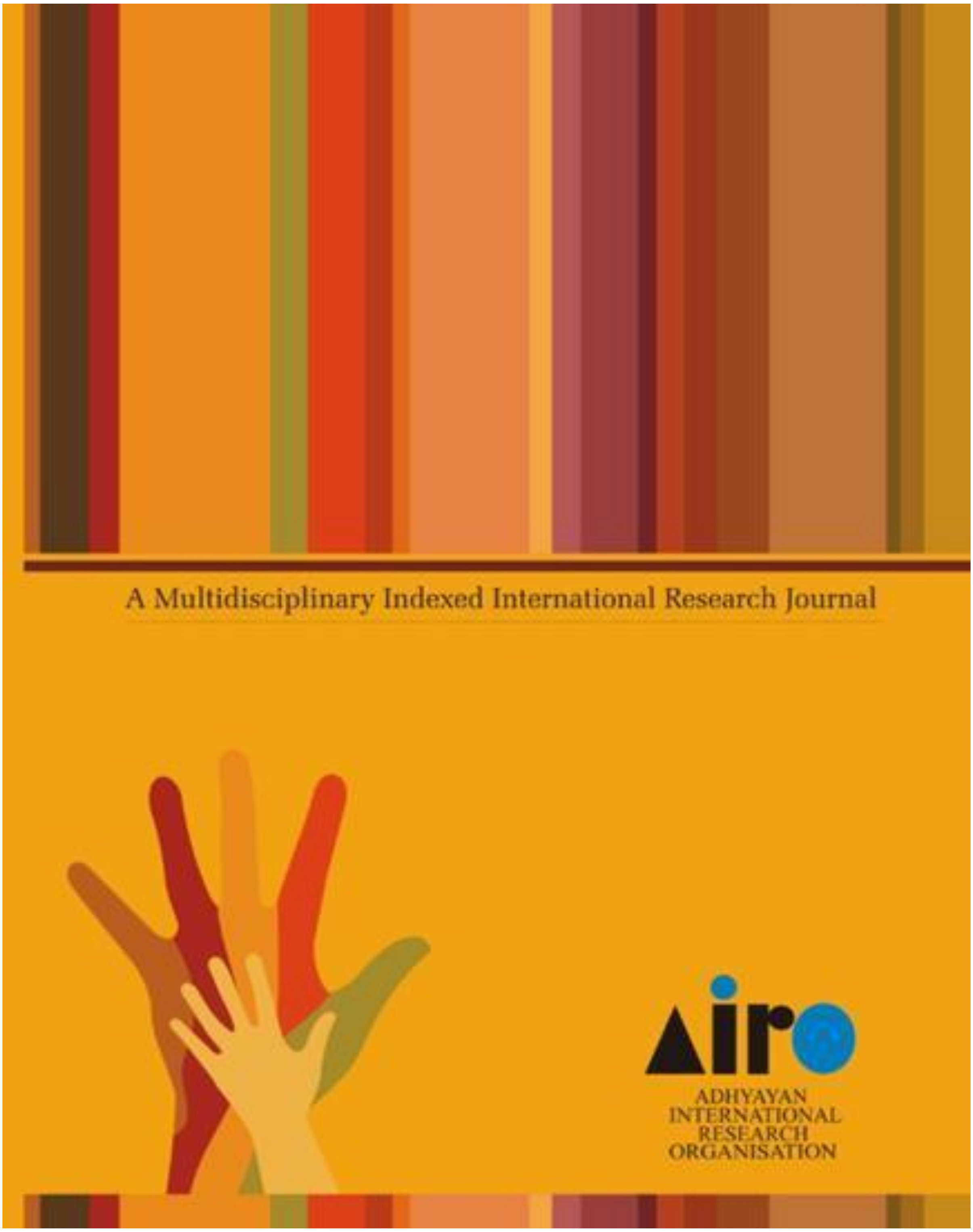

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EFFECT OF ZINC OXIDE NANO-PARTICLES ON THE MICROBES IN REFERENCE TO DIFFERENT ABIOTIC FACTORS

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Abstract: *With the rapid development of nanotechnology, nano-particles have been increasingly used in recent years. Zinc oxide nano-particles (ZnO-NPs) are common nano-particles and widely used in many fields such as sunscreen products, cosmetics, pigments, industrial coatings, plastic additives, semiconductors, textiles, and antibacterial agents . It was reported that the global production of nano-particles for sunscreen products was estimated to be approximately1000 tons during 2003-004, consisting principally of ZnO and TiO2 particles. The widespread production and use of ZnO-NPs increase the potential for their release to the environment. It was estimated that ZnO-NPs concentrations in the environment ranged from less than 100 µg·L–1(in water) to a few mg·kg–1 (in soil). Environmental levels of ZnO-NPs are expected to increase continually given the widespread and expanding applications of this material*

Keywords: *Nano-particles, Zinc oxide, antibacterial.*

INTRODUCTION

Nano-particle, ultrafine unit with dimensions measured in nano-metre (nm; 1 nm = 10^{-9} metre). Nano-particles exist in the natural world and are also created as a result of human activities. Because of their submicroscopic size, they have unique material characteristics, and manufactured nano-particles may find practical applications in a variety of areas, including medicine, engineering, catalysis, and environmental remediation.

Zinc oxide NPs has unique physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photostability is a multifunctional material. It has tremendous scientific and technological interest due to direct wide band gap energy (3.37 eV), large exciton-binding energy (60 meV) and high thermal and mechanical stability at room temperature make it attractive for potential use in electronics, optoelectronics and laser technology. The piezo- and pyroelectric properties of ZnO mean that it can be used as a sensor, converter, energy generator and photocatalyst in hydrogen production. Because of its hardness, rigidity and piezoelectric constant it is an important material in the ceramics industry, while its low toxicity, biocompatibility and biodegradability make it a material of interest for biomedicine and in pro-ecological systems.

REVIEW OF LITERATURE

Amma Sire Ikhatim (2015) Zinc oxide (ZnO) nano-particles (NPs) are manufactured worldwide in large quantities for use in a wide range of applications. ZnO NPs possess different physicochemical properties compared to their fine particle (FP) analogs, which might alter their bioactivity. Most of the literature cited here has focused on the respiratory system, showing the importance of inhalation as the primary route for ZnO NP exposure in the workplace. ZnO NPs may translocate to systemic organs from the lung and gastrointestinal tract (GIT) although the rate of translocation appears low. There have also been studies focusing on other potential routes of human exposure. Oral exposure mainly occurs through food products containing ZnO NP-additives. Most dermal exposure studies, whether *in vivo* or *in vitro*, report that ZnO NPs do not penetrate the stratum corneum (SC). In the field of nano-medicine, intravenous injection can deliver ZnO nano-particulate directly into the human body. Upon intravenous exposure, ZnO NPs can induce pathological lesions of the liver, spleen, kidneys, and brain. We have also shown here that most of these effects may be due to the use of very high doses of ZnO NPs. There is also an enormous lack of epidemiological data regarding ZnO NPs in spite of its increased production and use. However, long-term inhalation studies in rats have reported lung tumors. This review summarizes the current knowledge on the toxicology of ZnO NPs and points out areas where further information is needed.

Nowadays, ZnO Nano-particles are used in transparent coating of food as protection against the UV. ZnO is a bio-safe material that possesses photo-oxidizing and photocatalysis impact on chemical and biological species. ZnO particle bactericidal and bacterio-static mechanisms on generation of reactive oxygen species (ROS) including hydrogen peroxide (H2O2), OH- (hydroxyl radicals), and O2-2(peroxide).ROS has been a major factor for several mechanisms including cell wall damage due to ZnO-localized interaction, enhanced membrane permeability, internalization of NPs due to loss of proton motive force and uptake of toxic dissolved zinc ions. These have led to mitochondria weakness, intracellular outflow, and release in gene expression of oxidative stress which caused eventual cell growth inhibition and cell death. ZnO antibacterial bioactivity has been used in food packaging industry where ZnO-NPs are used as an antibacterial agent toward food borne diseases.

Giller (2008) the soil is considered as the land surface of the earth which provides the substratum for plant and animal life. The soil represents a favorable habitat for microorganisms and is inhabited by a wide range of microorganisms including bacteria, fungi, algae, viruses and protozoa. The physical structure, aeration, water holding capacity and availability of nutrients are determined by the mineral constituents of soil which are formed by the weathering of rock and the degradative metabolic activities of the soil microorganisms. Cultivated soil has relatively more population of microorganisms than the fallow land, and the soil rich in organic matter contain much more population than sandy and eroded soil. Microbes in the soil are important to us in maintaining soil fertility, cycling of nutrient elements in the biosphere and sources of industrial products such as enzymes, antibiotics, vitamins, hormones, organic acids etc. But certain microbes in the soil are the causal agents of various human and plant diseases.

Emamifar A (2011) the plant and animal remains deposited in the soil contributes organic substances. Soil micro organism's breakdown a variety of organic materials and use a portion of these breakdown products to generate or synthesize a series of compounds that make up humus, a dark colored amorphous substance composed of residual organic matter not readily decomposed by microorganisms. The three major fractions of humus are humic substances, poly-saccharides and other non-humic substances, and humin. These materials have impact on the physical, chemical and bio-chemical properties of soil in many ways. Humus improves the texture and structure of the soil, contributes to its buffering capacity and increase the water holding capacity of the soil.

Hekmatafshar M (2012) Zinc oxide nano-particles (ZnO-NPs) are increasingly released in agricultural soil through, e.g. bio solids, irrigation or nano-agrochemicals. Soil is submitted to a wide range of concentrations of ZnO-NPs depending on the type of exposure. However, most studies have assessed the effects of unrealistically high concentrations, and the dose–response relationships are not well characterized for soil microbial communities. Here, using soil microorganisms, we assessed the impact of ZnO-NPs at concentrations ranging from 0.05 to 500 mg kg^{-1} dry-soil, on the activity and abundance of ammonia-oxidizing archaea (AOA) and bacteria (AOB), and nitrite-oxidizing bacteria (*Nitrobacter* and *Nitrospira*). In addition, aggregation and oxidative potential of ZnO-NPs were measured in the suspension, as they can be important drivers of ZnO-NPs toxicity. After 90 days of exposure, non-classical dose–response relationships were observed for nitrifier abundance or activity, making threshold concentrations impossible to compute. Indeed, AOA abundance was reduced by 40% by ZnO-NPs whatever the concentration, while *Nitrospira* was never affected. Moreover, AOB and *Nitrobacter* abundance were decreased mainly at intermediate concentrations. Nitrification was reduced by 25% at the lowest (0.05 mg kg^{-1}) and the highest (100 and 500 mg kg^{-1}) ZnO-NPs concentrations. Studies have shown that ZnO-NPs affect nitrification through specific activity of nitrifiers, in addition to indirect effect on nitrifier abundances. Altogether these results point out the need to include very low concentrations of NPs in soil toxicological studies, and the lack of relevance of classical dose–response tests and ecotoxicological dose metrics (EC50, IC50…) for ZnO-NPs impact on soil microorganisms[14]. ZnO nano-particles which are mainly used as plastic fillers are based on two categories of organic and inorganic (e.g. Fe, Si, Ti, Ca, etc.) materials. The latter are more used in food packaging. ZnO nano-particles have been of interest to researchers in various disciplines thus far due to their physical, photocatalytic, low toxicity and allergic reaction properties. Nano-particles may work through different mechanisms; they can directly react with microbial cell or indirectly cause DNA damage of the microorganism. These nano-particles are most functional in self-cleaning and disinfecting by coating surfaces in food industry.

Studies showed that many organic compounds decompose to mineral compounds during this process. Hazardous organic compounds such as deodorants, pesticides, paint, etc. are the main pollutants that can affect the environment caused by means of pharmaceuticals, textiles and agriculture and food industries. Many of these toxins are capable of decomposing to harmless compounds (such as water and carbon dioxide) by photocatalytic reactions. Spoilage of dairy products is a huge economic problem as each year almost one fourth of food worldwide lost their nutritional value due to microbial activities.

PLAN OF WORK [MATERIAL AND METHODES]

Experimental design

Soil will be collected from different sites of the A.N College campus. Zinc oxide powder will be purchased from Eklingjee Polymers Pvt. Ltd.

Selection of microbes and diatom

The Lyophilized bacterial strains of Gram negative bacterium *E. coli* (ATCC8739), Gram positive bacterium *S .aureus*(ATCC6538) and fungus *A. flavus*(PTCC5004) will be selected for this study. And a diatom species such as *Stauroneis*sp will be chosen for the study.

Biosynthesis of Nano-particles

The development of simple and versatile methods for the preparation of nano-particles in a anisotropic size or shape selected and controlled manner has been a challenging but intellectually rewarding task. The nano-particles morphology often emerges as a result of a competitive growth of different crystallographic surfaces. This is typically achieved by altering the relative growth rates of different facets by the selective localization of surface-modifying or capping agents, but also by the modulation of nucleation and reaction parameters such as time, temperature, reagent concentration, and pH.

Analysis of zinc oxide nano-particles by electron microscopy

Scanning Electron Microscopy (Carl Zeiss, Germany) will be used for determination of diameter and surface area to volume ratio of nano-particles.

Microbial DNA extraction from soil for PCR amplification

Microbial DNA from soil will be extracted from 0.3 g of soil using a Power soil DNA isolation kit (MoBio, Carlsbad, CA) according to the manufacturer's instructions. Genes encoding 16S rRNA will be PCR-amplified using the extracted DNA as the template and a primer set well suited for bar-coded pyrosequencing.

Protease activity

Protease activity will be measured after 15 and 60 days to evaluate ENP effects on a specific soil microbial function that is critical to maintain soil N supply.

Antimicrobial effect of zinc oxide nano-particles

Two groups of samples will be considered for the evaluation of antimicrobial effect of nano-particles of zinc oxide and its photocatalyst type for each microorganism. The first group of samples (including four beakers) will be considered for evaluating the effect of ZnO nano-patrticles and the second group of samples (including five beakers) will be considered for evaluating the effect of photocatalyst type of nano-particles in the presence of UV light.

Statistical analysis

To test whether bacterial communities differed among treatments, the community dissimilarities will be illustrated through two complementary multivariate techniques, principal coordinate analysis (PCoA) and multivariate regression tree (MRT) analysis, and were statistically tested by nonparametric multivariate analysis of variance (NP-MANOVA) with 9,999 permutations.

OBSERVATIONS

Study on the effect of Zinc Oxide (ZnO) nano-particles against Staphylococcus aureus which is a pathogenic organism has been done. The antibacterial activity and the effect on growth and on protein level due to the interaction of nano-particles were observed from 20, 40, 60, 80 and 100 µg/mL concentrations. The results of the present study are given below. Antibacterial activity of ZnO Nano-particles against S. aureus From the results obtained due to the antimicrobial activity of ZnO nano-particles on S. aureus it was interesting to note that as the concentration of nano-particles increases, the zone of inhibition also increases i.e. a minimum for control (almost none) to a maximum in 100 µg/mL (Figure 1, Table 1).

Table 1 Antibacterial activity of ZnO Nano-particles against S. aureus

Zone Of Inhibition of S. aureus (mm) at various concentrations					
Control	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
0	11.26 ±1.23	11.26 ±1.23	13.41 ±1.67	13.46 ±1.53	14.52 ±2.01

Fig 1 Antibacterial activity of ZnO nano-particles against S. aureus (Kirby-Bauer test)

Colony Forming Units (CFU) in S. aureus when treated with ZnO Nano-particles The number of CFU of S. aureus has reduced significantly with increasing ZnO nano-particles. Minimum number of CFU observed in the control. Virtually no CFU were observed in the samples containing ZnO at the highest concentration i.e. 100 µg/mL. The bacterial growth inhibition trend found in CFU data has matched well with the results of optical density (Figure 2, Table 2)

Table 2 Colony forming units in S. aureus treated with ZnO nano-particles.

Colony forming units in S. aureus for different concentrations in ZnO nano-particles					
Control	**20 µg/mL**	**40 µg/mL**	**60 µg/mL**	**80 µg/mL**	**100 µg/mL**
43±4	24±3	12±2	7±2	6±2	2±1

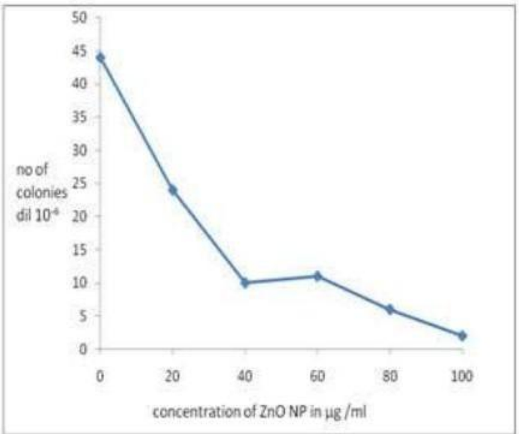

Fig 2 Graph - Colony Forming Units Concentration of NP vs. Number of colonies

CONCLUSION

The present study dealt with the biosynthesis of Zinc Oxide nano-particles using the root extracts of *S .aureus*(ATCC6538) that were characterized using Scanning Electron Microscope. These results reveal that the nanoparticle were spherical in shape and had an average size range of 30nm. The synthesized ZnO nanoparticles were checked for antibacterial activity which may be due to the reactive oxygen species produced by the nano-particle against bacteria as well as due to the electrostatic interaction between nano-particles and bacterial species. Moreover the size of the nano-particle also influenced the nano-particle's antibacterial activity. The ZnO nano-particle shows higher activity against gram negative bacterial strains than the gram positive strains. Thus, by using the greener method for producing ZnO nano-particle can have a potential impact on treating ailments pertaining to pathogenic bacteria and hence can be applied in pharmaceutics.

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