Original Research Article

The Effects of silicon dioxide nanoparticles and zinc oxide nanoparticles on Waste land soil bacterial and fungal isolates

ABSTRACT

Objective:

Different bacterial and fungal isolates were collected from wasteland municipality site, Tambaram. The antibacterial activity of two types of nanoparticles [Zinc oxide and Silicon dioxide] ZnO & SiO2 against several types of Gram-negative bacteria and fungi was investigated in this work.

Methods:

P. aeruginosa, B. subtilis, Pencillium oxalicum and *Aspergillus fumigatus* were isolated from 10 soil samples taken from three sites of Tambaram Municipality waste land (Chennai). After collecting the samples, we used culturing and biochemical tests to identify the microbes, and then used a chemical approach to make ZnO and SiO_2 nanoparticles with altered structure and morphological features. Minimum inhibitory concentration (MIC) was used to assess the antibacterial activity of these nanoparticles against various microorganisms.

Results:

The best inhibition zone was found in *Psuedmonas sps* and *Bacillus sps* growth at concentrations of 10 µg/ml and 5 µg /ml of nano-ZnO, respectively, whereas the lower inhibition zone was found in *Pencillium oxalicum* and *Aspergillus fumigatus* at a dosage of 2.5 µg /ml of the same nano particle. It was also discovered that no inhibitory zone existed in any of the bacteria and fungi at a concentration of 10 µg /ml nano-SiO_2. We found that all of the bacteria and fungi we tested were completely inhibited at a concentration of 1.25 g/ml nano-ZnO (MIC), with no antibacterial activity below this concentration. When compared to data that showed that

all tested bacteria were not completely inhibited even at a concentration of 0.625 g/ml of nano-SiO_2.

Conclusion:

In comparison to the two nanoparticles (ZnO and SiO2), nano-ZnO outperformed nano-SiO2 in inhibiting most bacteria and fungi at the quantities tested in waste land soil.

Key words: Zinc oxide nanoparticles, soil sample, Silicon dioxide nanoparticles, bacteria, fungi and Minimum inhibitory concentration.

INTRODUCTION

Today's municipal solid waste constitutes a huge environmental danger. Traditional dumping tactics are used for waste management in poor countries since MSW treatment processes are not sufficiently established.

In order to prevent microbial infections from the soil, new researchers are interested in exploring novel compounds with distinctive properties at the atomic, molecular, and macromolecular levels. [1] Most biologists are now focusing their study on the utilisation of this substance because of its efficiency in inhibiting resistant pathogenic strains due to its chemical, physical, and toxicological stability, as well as their anticancer impact in humans. [2,3]

The rise in bacterial diseases by waste land soil exist throughout the world, as well as the introduction of novel pathogenic strains and increased soil toxicity, has prompted researchers to investigate the properties of these new substances and their effects on altering bacterial growth like shape, concentrations and other morphological properties. [4]

Nanoparticles have shown antibacterial action when they connect with bacterial surfaces and subsequently penetrate the cell, destroying it. These materials may have bactericidal properties that are useful in a variety of antimicrobial applications, just as they are in industry. [5] Plant growth regulators, antibacterial activities, and plastic degraders all benefit from the usage of nano-particles ZnO and SiO2 at precise concentrations.

The harmful action of this substance is related to the oxidative stress (OS) that destroys lipids, carbohydrates, proteins, and DNA, which affects the cell wall and membrane of bacterial cells. Many studies have found that nanoparticles with higher concentrations and greater surface areas have stronger antimicrobial action. [6,7] They also demonstrated the structure and circumstances of various culture methods, as well as their impact on the physicochemical and biological characteristics of nanoparticles, as well as their toxicity, when pH and temperature were varied.

The study of the influence of nano-particles on various microbial growth is significant for biologists because of their antibacterial and unique activity in biological sciences, particularly at the nano-scale, against a wide range of micro-organisms such as bacteria, fungus, fish, algae, and plants. [8]

The pathogenic bacteria and fungi cause persistent infections that are difficult to cure because of their propensity to harm host tissues, resulting in antibiotic resistance and death. As a consequence, scientists have identified novel ways for controlling microbial infections caused by natural or inorganic compounds that will be employed in the future generation of medications or agents.

The goal of this research is to test the antibacterial activity [*in vitro*] of two types of nanoparticles (ZnO and SiO2) against gram-negative bacteria and fungi.

Materials and Methods

METHODOLOGY AND RESEARCH DESIGN:

This is a prospective study conducted in the department of microbiology, Sri Akilandeswari college for women, Wandiwash.

SAMPLE COLLECTION:

Three Soil samples were collected from waste disposal site of Tambaram Municipality. The samples were sieved (mesh size < 2 mm) to remove stones and dust debris.

Isolation of Microorganisms:

1g of soil sample was added separately in 9 ml sterile saline and serially diluted. $10^{-5}$and 10^{-6} dilutions were plated on nutrient agar and incubated at 37^{o} C for 24-48 hours to isolate different bacterial strains.10^{-3} and 10^{-4} dilutions were plated on Potato dextrose agar with Chloramphenicol and incubated for 48 hours to isolate different fungal strains. The colonies with different colony morphology were selected and sub cultured in the respective media for further use.

Identification of microorganisms

The isolated organisms were screened based on their ability to utilize polyethylene as sole carbon source. Bacteria were identified on the basis of microscopic examination and biochemical analysis according to McCarteny, Practical Medical Microbiology. [9] Fungi were identified based on colonial morphology and microscopic appearance by using Lactophenol cotton blue staining.

Nanoparticle preparation:

SiO_2 NP powder (<50 nm) and Zn O was purchased from Sigma Aldrich (Chennai). Both Zn O and SiO2 nanoparticles (approximately 0.02 gm) were dissolved in (10 ml) of dimethyl sulfoxide (DMSO) to make a stock solution of (1 mg/ml), and then (1 ml) of these solutions was diluted in (10 ml) of DMSO to make a solution with a concentration of (100 g/ml). This solution was used to make the concentrations that were tested, which included: For the determination of the minimum inhibitory concentration (MIC), 10, 5, 2.5, 1.25, 0.625 & 0.312 µg/ml were created. [10]

Minimum inhibitory concentration (MIC) and Minimum Bactericidal concentration:

The lowest inhibitory doses in nano-ZnO and nano-SiO2 and against several bacterial and fungal isolates were determined according to. [11] The MIC is the lowest concentration that prevents the bacterium and fungi under investigation from growing. In this experiment, 1 ml of medium (nutrient broth) was placed in a test tube, to which 1 ml of test solution was added, followed by 0.1 ml of bacterial isolates produced in 0.9 percent NaCl being added to the test tube holding the media and test solution. Each nano-particle was serially diluted six times, yielding concentrations of (10 – 5 – 2.5 – 1.25 – 0.625 & 0.312) µg/ml. At 37°C, all test tubes were incubated for 24 hours.

The presence of turbidity was used to determine the affirmative result, which was compared to a sample of 0.5 McFarland standards. We discovered that inoculated broth samples containing only DMSO at the same dilutions employed in this study had no influence on bacterial growth in the control test. The MIC values were determined by subculturing 50µg/ml from each test group, which showed no clear growth after incubation (no turbidity).

Experimental design and statistical analysis

The one-way ANOVA in SPSS was used to test MIC test (P-value = 0.05).

Results and Discussion:

A total of 3 soil samples were collected from waste disposal site of Tambaram Municipality. Bacteria and fungi were isolated and identified from soil samples. Identified bacteria and fungi were tabulated below (table: 1) In the same vein Sairy et al., 2014 assessed soil sample from municipal site for isolation of mesophilic, thermophilic bacteria and fungi for enzymatic testing. [12] Same like Murugesan et al., 2020 collected soil sample from vegetable market complex waste from Tambaram Municipality (Latitude: 12.9229°N, Longitude: 80.1275° E) located in Chennai, Tamil Nadu, India. It is reported that the solid waste generated from Tambaram Municipality requires 19.27 acres of landfill. [13]

Bacteria and Fungi were capable of growing in the waste polluted soil. Many of the isolated fungi, such as *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus* are inhabitants of soil and plant decayed matter organisms [14]. Like wise bacteria like *Psuedomonas sps and Bacillus sps* are present in large numbers, due to the solid waste and timely release of wastewater (effluents) into the soil environment, which may cause infections to humans from the soil.

This study corroborated earlier studies [15,16] that reported isolation of Aspergillus, Penicillium and Bacillus sp in soil contaminated with effluent isolated. In addition, Penicillium oxallicum were reported in soil laden with waste water effluents

Table 1. Identification of Bacterial isolates from soil sample:

Isolate	Identified microbes
JSB 1	*Bacillus subtilis*
JSB 2	*Psuedomanas aeruginosa*
JSF 3	*Pencillium oxalicum*
JSF 4	*Aspergillus fumigatus*

Minimum Inhibitory concentrations of Zn O and SiONPs

Our results have explained the antimicrobial activity of nano-ZnO & nano-SiO_2 suspensions against different types of pathogenic bacteria and fungi. We use six nano-ZnO & nano-SiO_2 suspensions with different concentrations that are tested of (10, 5, 2.5, 1.25, 0.625 & 0.312 µg/ml) as in table 2, the data show, The best inhibition zone was found in *Psuedmonas sps* and *Bacillus sps* growth at concentrations of 10 µg/ml and 5 µg /ml of nano-ZnO, respectively, whereas the lower inhibition zone was found in *Pencillium oxalicum* and *Aspergillus fumigatus* at a dosage of 2.5 µg /ml of the same nano particle.

This is happened because these nano-particles have the ability to penetrate the bacteria cell walls and cause damage to its cytoplasm which leads to bacteria inhibition. Nano-particles ZnO have the ability to release free radical like: hydrogen peroxide (H_2O_2), OH- (hydroxyl radicals), and O_2 (peroxide) into the medium after the surface of the dead bacteria is completely covered by nano-particles to prevent any bacterial action, so it shows high bactericidal efficacy. [17]

By interfering with cell activity and inducing distortion in fungal hyphae, ZnO NPs stop fungus from growing. In contrast, ZnO NPs with concentrations more than 6 m/mol completely suppress the growth of *Pencillium oxalicum* and *Aspergillus fumigatus* by blocking the production of conidiophores and conidia. [18]

It was also discovered that no inhibitory zone existed in any of the bacteria and fungi at a concentration of 10 µg /ml nano-SiO2. We found that all of the bacterial and fungal, we tested were completely inhibited at a concentration of 1.25 µg /ml nano-ZnO (MIC), with no antimicrobial activity below this concentration.

This have explained that there are many differences between our results and the results of other researches [19,20] who suggested higher antimicrobial efficacy of Silicon dioxide nanoparticles and this has occurred due to several factors like: methods of isolation of clinical bacterial strain that may form effective biofilm, difference in the preparation methods of nano-particles, size and concentrations of tested nano-particles in addition to the types of culture media and other parameters induced the bacterial and fungal growth

Table: 2 MIC values of zinc oxide and Silicon dioxide nanoparticles against bacteria and fungi.

Identified Bacteria	**MIC µg/ml**	
	ZnO	**SiO_2**
Bacillus subtilis	5 [0.05]	<0.625 [p=0.21]
Psuedomanas aeruginosa	10 [0.04]	
Pencillium oxalicum	2.5 [0.05]	
Aspergillus fumigatus	2.5 [0.05]	

Conclusions

Our results have shown that the Compare to SiO_2 nanoparticles, ZnO nanoparticles have antibacterial activity and could inhibit most of the important pathogenic bacteria (G^-) at the tested concentrations that are isolated from waste land soil. Silicon dioxide, on the other hand, is harmless to isolated bacteria.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Dušan Z, Vladimir V.S, Maja A.K & Milan N.M. Antimicrobial properties of ZnO nanoparticles incorporated in polyurethane varnish. *Processing and Application of Ceramics.* 2011;5[1]:41–45.
2. Zarrindokht E.K & Pegah C. Antibacterial activity of ZnO nanoparticle on gram positive and gram-negative bacteria. *African Journal of Microbiology Research*. 2011;5(12):1368-1373.

3. Rizwan W, Nagendra K.K, Akhilesh K.V, Anurag M, Hwang I.H, You-Bing Y, Hyung-Shik S.h & Young-Soon K. Fabrication and growth mechanism of ZnO nanostructures and their cytotoxic effect on human brain tumor U87, cervical cancer HeLa, and normal HEKcells. *J. Biol. Inorg. Chem*. 2010;16(3):431-442.
4. Aysa N.H & Salman H.D. Antibacterial activity of modified zinc oxid nanoparticles against *Pseudomonas aeruginosa* isolates of burn infections. *WSN.* 2016;33:1-4.
5. Amna S, Shahrom M, Azman S,Noor H, Mohamad K, Ling C. A, Siti K, Mohd B, Habsah H& Dasmawati M. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-Micro Lett*. 2015;7(3):219–242.
6. Ivanova I.A, Tasheva-Terzieva E, Angelov O, Krusteva L, Andonova I, Papazova K Dimova-Malinovska D & Dushkin C. Effect of ZnO thin films on survival of *Pseudomonas* cells. *J Nanomed Nanotechol.* 2012;3(7):2-7.
7. Li M, Pokhrel S, Jin X, Mädler L & Damoiseaux R. Stability, bioavailability, and bacterial toxicity of ZnO and iron-doped ZnO nano particles in aquatic media. *Environ Sci Technol.* 2011;45:755-761.
8. Saliani M, Jalal J & Kafshdare E.G. Effects of pH and temperature on antibacterial activity of zinc oxide nano fluidagainst *Escherichia coli* O157: H7 and *Staphylococcus aureus*. *Jundishapur J Microbiol*. 2015;8(2):e17115.
9. Collee J.G, Fraser A.G, Marmiom B.P & Simmon A. Mackie and McCarteny, Practical Medical Microbiology. 4*th* ed. *Churchill Livingstone Inc*. USA. 1996.
10. Govinda R.N, Thripuranthaka M, Dattatray J.L & Sandip S.S. Antimicrobial activity of ZnO nanoparticles against pathogenic bacteria and fungi. *JSM Nanotechnol Nanomed.* 2015;3(1):1033.
11. Sangani M.H, Moghaddam M.N & Forghanifard M.M. Inhibitory effect of zinc oxide nanoparticles on *pseudomonas aeruginosa* biofilm formation. *Nanomedicine Journal*. 2015;2(2):121-128.
12. Bin Saleh, Sairy Abdullah & Mohammed, Asef Iqbal & Fazil, Mohammed. (2014). Isolation of Bacterial and Fungal cultures for Effective aerobic composting of Municipal solid waste. 1. 59-62. 10.13140/2.1.3687.9040.

13. Murugesan V, Amarnath DJ. Bio-process performance, evaluation of enzyme and non-enzyme mediated composting of vegetable market complex waste. Scientific reports. 2020 Nov 13;10(1):1-2.
14. D., Mohammed. (2020). Screening and molecular identification of Fungi isolated from soil with potential for bioremediation of tannery waste polluted Soil. 7. 6-15.
15. Aysa N.H, Al-Maamori M.H & Al-Maamori N.A.A. Effect of the unmodified and modified ZnO nanoparticles on the mechanical and antibacterial properties of silicone rubber using in medical applications. *Journal of Nanoscience and Nanoengineering.* 2015*;*1(3):119-124.
16. Jiang J.O.G, Elder A, Gelein R, Mercer P, Beswas A. Dose nanoparticle activity depend upon size and crystal phase ? *Nanotoxicology*. 2008;2:33-42 . CrossRef
17. Thati V, Roy A.S, Prasad M.V.N.A, Shivannavar C. T &Gaddad S.M. Nanostructured zinc oxide enhances the activity of antibiotics against *Staphylococcus aureus*. *J Biosci Tech.* 2010;1(2):64-69.
18. Amna S, Shahrom M, Azman S,Noor H, Mohamad K, Ling C. A, Siti K, Mohd B, Habsah H& Dasmawati M. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-Micro Lett*. 2015;7(3):219–242. CrossRef
19. Ivanova I.A, Tasheva-Terzieva E, Angelov O, Krusteva L, Andonova I, Papazova K Dimova-Malinovska D & Dushkin C. Effect of ZnO thin films on survival of *Pseudomonas* cells. *J Nanomed Nanotechol.* 2012*;*3(7):2-7.
20. Kadhum SA. The Effect of two Types of Nano-Particles (ZnO and SiO2) on Different Types of Bacterial Growth. Biomedical & Pharmacology Journal. 2017;10(4):1701.