



The Effects of Silicon Dioxide Nanoparticles and Zinc Oxide Nanoparticles on Waste Land Soil Bacterial and Fungal Isolates

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

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

Methods: *P. aeruginosa*, *B. subtilis*, *Penicillium oxalicum* and *Aspergillus fumigatus* were isolated from 5 soil samples taken from three sites of Tambaram Municipality wasteland (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 SiO₂ 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 *Pseudomonas 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 *Penicillium oxalicum* and *Aspergillus fumigatus* at a dosage of 2.5 µg /ml of the same nanoparticle. 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 bacteria and fungi we tested were completely inhibited at a concentration of 1.25 g/ml nano-ZnO (MIC),

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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-SiO₂.

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 wasteland soil.

Keywords: Zinc oxide nanoparticles; soil sample; Silicon dioxide nanoparticles; bacteria; fungi and Minimum inhibitory concentration.

1. 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 utilization of this substance because of its efficiency in inhibiting resistant pathogenic strains due to its chemical, physical, and toxicological stability, as well as its anticancer impact in humans [2,3].

The rise in bacterial diseases by wasteland soil exists 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 the 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 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 nanoparticles on various microbial growth is significant for biologists because of their antibacterial and unique activity in biological sciences, particularly at the nanoscale, against a wide range of microorganisms 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 antimicrobial activity [*in vitro*] of two types of nanoparticles (ZnO and SiO₂) against gram-negative bacteria and fungi.

2. MATERIALS AND METHODS

2.1 Methodology and Research Design

This prospective study was conducted in the Department of Microbiology, Sri Akilandeswari women's college, Wandiwash.

Sample collection: Three Soil samples were collected from the 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 37⁰ 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 subcultured 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 McCartney, Practical Medical Microbiology [9]. Fungi were identified based on colonial morphology and microscopic appearance by using Lactophenol cotton blue staining.

Nanoparticle preparation: SiO₂ NP powder (<50 nm) and Zn O were 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 nanoparticle was serially diluted six times, yielding concentrations of (10 – 5 – 2.5 – 1.25 – 0.625 & 0.312) µg/ml. At 37°C, the microtiter plate was incubated for 24 hours. Each organism were tested twice a time in microtiter plate.

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).

3. RESULTS AND DISCUSSION

A total of 5 soil samples were collected from the waste disposal site of Tambaram Municipality. Bacteria and fungi were isolated identified from soil samples. Identified bacteria and fungi were tabulated below (Table 1) In the same vein Sairy et al., 2014 assessed soil samples from the municipal site for isolation of mesophilic, thermophilic bacteria and fungi for enzymatic testing [12]. Same like Murugesan et al., 2020 collected soil samples 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 could grow 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]. Likewise, bacteria like *Pseudomonas 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 the isolation of *Aspergillus*, *Penicillium*, and *Bacillus sp* in soil contaminated with effluent isolated. In addition, *Penicillium oxalicum* were reported in soil laden with wastewater effluents

Biochemical identification of different bacteria and fungi were tabulated below.

Different Colonies on the PDA plate of fungi were done with LPCB staining and KOH mount.

Morphological Characteristics were identified under staining. Identified fungi were listed below in Table: 2.

Table 1a. Identification of different bacteria from soil sample

Isolate	Gram Reaction	Spore former	Motility	Morphology	Indole	Methyl Red	VP	Citrate	TSI	Urease
JSB 1	+	+	-		Rods	+	-	+	-	-
JSB 2	+	-	+		Rods	-	-	+	-	-

Table 1b. Sugar fermentation test of different Bacteria from soil sample

Isolate	Arabinose	Fructose	Galactose	Glucose	Lactose	Maltose	Xylose	Identification of Genus
JSB 1	-	+	+	+	-	+	-	<i>Bacillus sp</i>
JSB 2	-	-	-	+	-	+	-	<i>Pseudomonas sp</i>

Table 2. Identification of Different Fungi from soil sample

Strain number	Colony Characterization	LPCB staining	Identified fungi
JSF 1	Velvety, downy or powdery, showing various shades of green colonies seen	Subclavate vesicles and hyphae seen	<i>Aspergillus sps</i>
JSF 2	flat, filamentous, and velvety, woolly, or cottony in texture	Hyphae and fruiting structures seen	<i>Penicillium sps</i>

3.1 Minimum Inhibitory Concentrations of Zn O and SiONPs

Our results have explained the antimicrobial activity of nano-ZnO & nano-SiO₂ suspensions against different types of pathogenic bacteria and fungi. We use six nano-ZnO & nano-SiO₂ suspensions with different concentrations that are tested of (10, 5, 2.5, 1.25, 0.625 & 0.312 µg/ml) as in Table 2 and Fig. 1, the data show, The best inhibition zone was found in *Pseudomonas 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 *Penicillium oxalicum* and *Aspergillus fumigatus* at a dosage of 2.5 µg /ml of the same nanoparticle.

This happens because these nanoparticles have the ability to penetrate the bacteria cell walls and cause damage to its cytoplasm which leads to bacteria inhibition. Nanoparticles ZnO have the ability to release free radicals like hydrogen

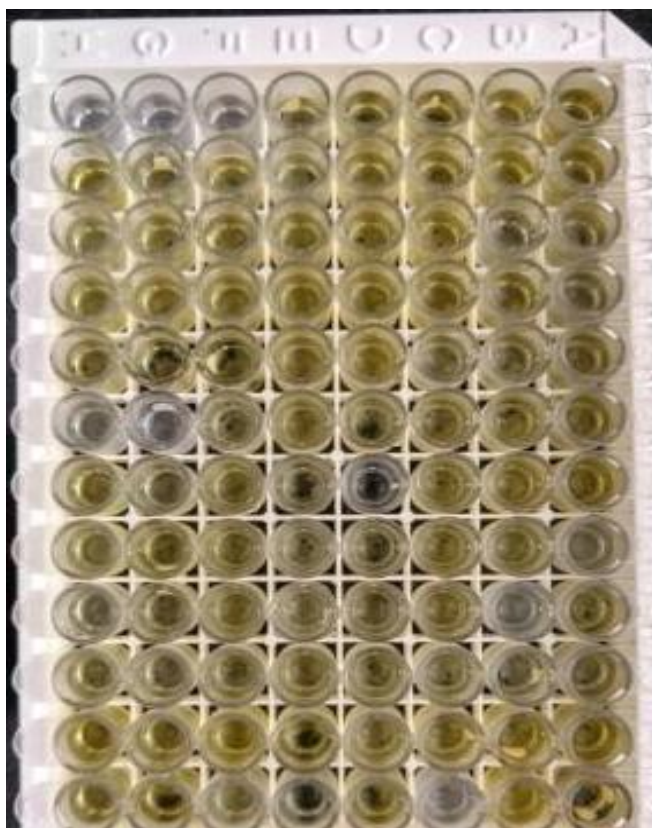
peroxide (H₂O₂), OH⁻ (hydroxyl radicals), and O₂ (peroxide) into the medium after the surface of the dead bacteria is completely covered by nanoparticles to prevent any bacterial action, so it shows high bactericidal efficacy [17].

By interfering with cell activity and inducing distortion in fungal hyphae, ZnO NPs stop fungus from growing. In contrast, ZnO NPs with concentrations of more than 6 m/mol completely suppress the growth of *Penicillium 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 isolates, we tested were completely inhibited at a concentration of 1.25 µg /ml nano-ZnO (MIC), with no antimicrobial activity below this concentration.

Table 3. MIC values of zinc oxide and Silicon dioxide nanoparticles against bacteria and fungi

Identified Bacteria	MIC $\mu\text{g/ml}$	
	ZnO	SiO ₂
<i>Bacillus subtilis</i>	5	
<i>Pseudomonas aeruginosa</i>	10	
<i>Penicillium oxalicum</i>	2.5	<0.625
<i>Aspergillus fumigatus</i>	2.5	

**Fig. 1. Broth dilution technique**

This has explained that there are many differences between our results and the results of other researchers [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 an effective biofilm, the 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

4. CONCLUSION

Our results have shown that Compare to SiO₂ nanoparticles, ZnO nanoparticles have antimicrobial activity and could inhibit most of the

important pathogenic bacteria and fungi at the tested concentrations that are isolated from wasteland soil. Silicon dioxide, on the other hand, is harmless to isolated microbes.

DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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