

CHEMICALLY SYNTHESIZED ZnO AND Al, Ag AND Cd DOPED ZnO NANOPARTICLES AT HIGHER TEMPERATURE 600°C, STUDY OF ANTIBACTERIAL ACTIVITY

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Abstract:

Sol-Gel chemical precipitation method has been implemented to synthesize ZnO nanoparticles at 600 °C. Wurtzite hexagonal crystal structure of as prepared pure and Al, Ag and Cd doped ZnO 600°C nanoparticles have been confirmed by X-ray Diffraction (XRD) data. The crystalline size and have been estimated from XRD data. The chemical composition of as prepared ZnO and 5% Al, Ag and Cd doped ZnO at 600°C samples nanoparticles has been investigated by analysis of Fourier transform infra red spectroscopy (FTIR). The direct band gap of nanoparticles has been estimated using UV absorption spectra. The effect of calcination temperature of Chemically prepared pure ZnO and 5% Al, Ag and Cd doped ZnO at 600°C have been comparatively study using *Bacillus thuringiensis* NCIM2130 and *Pseudomonas cf. monteilii* 9 culture.

Keywords: Sol – Gel precipitation, ZnO nanoparticles, XRD, SEM, FTIR, UV, *Bacillus thuringiensis* NCIM2130 and *Pseudomonas cf. monteilii* 9 Antibacterial activity.

Introduction:

Zinc Oxide (ZnO) is one such important metal of multi dimension characteristics use. It has the unique optical and electrical properties which can be used in a variety of applications, such as high transmittance conductive oxide coatings for solar cells, gas sensors, UV photodetectors, and bulk acoustic wave resonators. It has direct bandgap energy of 3.37 eV, which makes it transparent in visible light and operates in the UV to blue wavelengths. The exciton binding energy is 60 meV for ZnO. The higher exciton binding energy enhances the luminescence efficiency of light emission [2, 3]. ZnO has exhibited better radiation resistance for possible devices used in space and nuclear applications [4]. Furthermore, ZnO is bio-safe and biocompatible, and may be used for biomedical applications [5].

ZnO has also been confirmed as a promising functional material in other nano devices such as field emitters and gas sensors. The synthesis of ZnO nanostructures is thus currently attracting intense worldwide interest. Other favorable aspects of ZnO include its broad chemistry leading to many opportunities for wet chemical etching, low power threshold for optical pumping, radiation hardness and biocompatibility. For a semiconductor to be useful, particularly in reference to optoelectronic devices; band gap engineering is a crucial step in device development. In the case of ZnO, alloying with MgO and CdO is an effective means of increasing or decreasing the energy band gap respectively.

The main advantage of zinc oxide nanostructures and is the wide band gap that can be utilized in various electronic and optoelectronic devices. Therefore we planned to synthesis of different type's morphology of pure and doped ZnO nanostructures and study their structural and spectroscopic properties. The antibacterial activity of pure and doped ZnO nanostructural have been studied.

(Fakhroueian *et al.*, 2013). Different methods have been used for the production of ZnO nanoparticles such as 1) Chemical synthesis 2) Hydrothermal method 3) Electrophoretic deposition 4) Co-precipitation 5) Mechanochemical-thermal synthesis 6) Chemical vapour depositions 7) Thermal decomposition 8) Sol-Gel method 9) Electrochemical depositions and 10) Anodization

The present study was aimed to i) Synthesis ZnO nanoparticle using simple Sol – Gel chemical precipitation method by utilizing Zinc acetate and sodium hydroxide in distilled water, ii) study the morphological changes after calcinations at 600°C temperature and iii) detect the antibacterial activity of ZnO nanoparticles.

In order to study the antibacterial activity of doped ZnO nanoparticles against *Bacillus thuringiensis* NCIM2130 and *Pseudomonas cf. monteilii* 9 cultures slightly modified Agar Well Diffusion method was used. The pure ZnO and Al doped ZnO, Ag doped ZnO, and Cd Doped ZnO nanopowder were calcined at 600 °C and used for antibacterial study.

Experimental method: Sol-Gel Chemical precipitation method for synthesized ZnO nanoparticles:

The chemicals used for this work were of analytical grade obtained from Merck (Mumbai).

A modified method of Fakhroueian *et al.*, 2013 was used where Zinc acetate and sodium hydroxide and distilled water used in the preparation of ZnO nanoparticles. Zinc acetate and sodium hydroxide was added slowly drop wise in a molar ratio of 1:2 under vigorous stirring, and stirring was continued for 4 hours. First Zinc acetate stirred for 2 hours and same time sodium hydroxide for 2 hours. Then the mixture of zinc acetate was slowly added drop wise in the solution of sodium hydroxide by continuous string for 2 hours. The precipitate obtained was filtered. The precipitate was dried in an oven at 100°C and ground to fine powder using agate mortar. The powder obtained from the above method was calcinated different temperatures such as prepared pure ZnO 600°C for 2 hours. Similarly method used for 5% doping Al, Ag and Cd in ZnO. The prepared pure sample ZnO 600°C and doped ZnO(Al)

600°C, ZnO(Ag)600°C and ZnO(Cd) 600°C was then characterized for physical and morphological changes by X – ray diffraction (XRD), UV absorption spectra, Scanning Electron Microscopy (SEM) and FTIR. **Results and Discussion:**

1.X - Ray Diffraction pattern study for prepared ZnO and doped ZnO(Al,Ag and Cd) 600°C:

The X – ray diffraction (XRD) data were recorded by the intensity data collected over a 2θ range of 20-80 degrees. The particle size of samples was estimated with the help of Debye-Scherrer's equation using the full width at half maximum of (100)(002) and (101) of the x-ray diffraction peaks (as shown in Figure 1). The particle crystalline size increases with increase in calcination temperature. A significant increase in crystalline size is observed for the sample calcination of as prepared ZnO600°C and doped ZnO(Al)600°C, ZnO(Ag) 600°C, and ZnO(Cd)600°C.

$$D = 0.94 \lambda / \beta \cos \theta \quad \text{..... (1)}$$

Where D is size of crystalline nanoparticles(nm), λ is wavelength of incident X – ray (nm) β is the full width at half maximum and θ is the diffraction angle.

X- ray diffraction studies confirmed that the synthesized ZnO all the diffraction peaks with the report (Origin 8.0 software) data and no characteristics were observed other than ZnO. The mean grain size of the particles was determined from the XRD line broadening measurement using Debye-Scherrer equation. The diffraction peaks indicates that the synthesized material was in nanometer range. The particle size was found to be in the range 48.73nm depending on the calcination condition (as shown in Table 1). The lattice parameters calculated were also in agreed with report values. Similarly procedure used to calculate particle size doped ZnO(Al,Ag and Cd) (44.18,46.36 and 41.54nm).The reaction temperatures greatly influence the particles morphology of prepared ZnO nanoparticles. The size of ZnO nanoparticles increased as the temperature for the hydrothermal synthesis increases. This is due to the change of growth between the different crystalline planes.

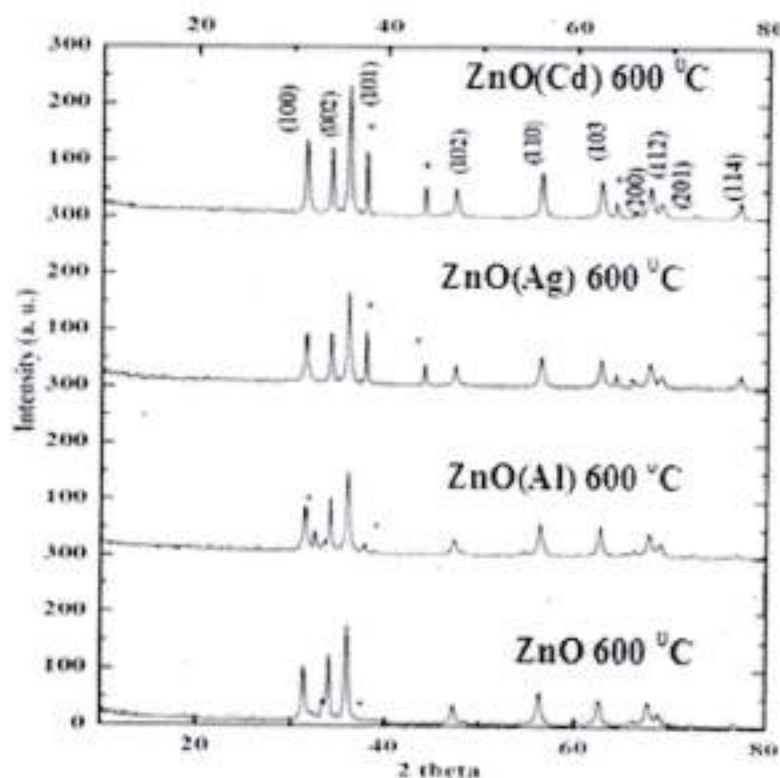


Figure 1: Shows XRD pattern of as prepared ZnO and doped ZnO(Al,Ag and Cd) at 600°C

Table 1: FWHM, Particles Size, (h,k,l) Lattice constant of ZnO and Al, Ag and Cd Doped ZnO nanoparticles.

ZnO Sample	2 Theta 2θ	d (Å)	h k l	FWHM (deg)	Particles Size D(nm)	Lattice Parameters	
						'a'	'c'
ZnO	31.7942	2.8122	100	0.3446		3.24	5.205

600°C	34.4443	2.6016	002	0.3078	48.73		
	36.2783	2.4742	101	0.3611			
	47.7489	1.90480	102	0.2952			
ZnO (Al) 600°C	31.4975	2.84039	100	0.3149	44.18	3.181	5.250
	34.1553	2.62520	002	0.3149			
	35.9835	2.49591	102	0.3149			
ZnO (Ag) 600°C	31.9616	2.80020	100	0.2952	46.36	3.284	5.255
	34.6379	2.58973	002	0.3936			
	36.4400	2.46568	101	0.3936			
	47.7176	1.90598	102	0.3936			
ZnO (Cd) 600°C	31.5720	2.83385	100	0.4723	41.54	3.272	5.231
	34.3536	2.61789	002	0.3149			
	36.0813	2.48937	101	0.3149			
	47.3652	1.91933	102	0.3149			

2. Scanning Electron Microscopic Study (SEM):

The Scanning Electron Microscopic images (SEM) of the samples also shows that (Figure.2) the physical structure of ZnO was changed with calcinations temperature. The samples calcinate at 600°C. The SEM images of ZnO samples show that the agglomeration particles are much less in this method of preparation. High resolution SEM images of ZnO calcinate show the presence of nanoparticles grain size 52nm. The SEM images of Al doped ZnO-NPs were shown in figure.2 exhibits distinct, inhomogeneous porous network with an average grain size 66 nm which is estimated from visualized data in micrographs. The wurzite hexagonal shaped nano rods of Ag attach on ZnO nanoparticles grain size 69nm. The small crystalline agglomerated to form nano fused clusters seen in the surface morphology of ZnO(Ag). The microstructure was observed changes to hexagonal nanorods at high temperature. The size of nanorods and nano grains are vary from 42 to 69nm which is visualized in SEM images. The increase in grain size as compared to obtained from XRD data is due to agglomeration of the grains together. ZnO(Cd) nanostructures exhibit significant varying morphology and may be used as antibacterial agent grain size 61nm.

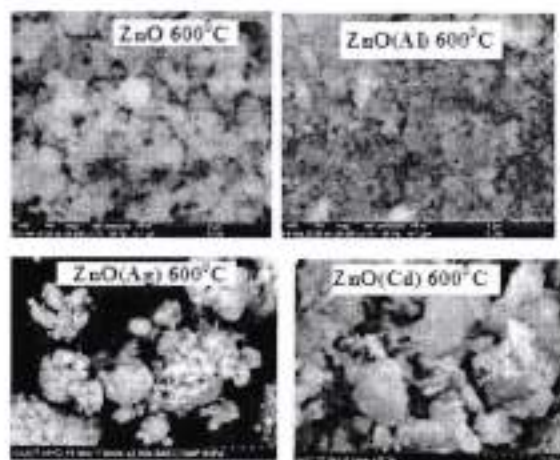


Figure 2. SEM image shows morphology ZnO Nanoparticles and ZnO (Al, Ag and Cd) doped Nanoparticles at 600°C.

Tables No.2 Grain Size ZnO nanoparticles from SEM	
ZnO and ZnO Doped Samples,	Grain Size from SEM (nm)
ZnO 600°C	52
ZnO(Al) 600°C	66
ZnO(Ag) 600°C	69
ZnO(Cd) 600°C	61

3. FTIR Study of As-prepared Pure ZnO and calcined ZnO(Ag)

FTIR spectra of prepared and calcined ZnO were recorded at 600°C temperature in the wave number range 399-4000 cm⁻¹ and presented in figure 3. The modes of vibrations and their band assignment were shown in table 3. FTIR spectra of the ZnO

nanopowder exhibit several characteristics bands with remarkable features. The absorption band assigned in the region 447 to 470 cm^{-1} attributed to characteristics stretching mode of ZnO and Al-O bonds [22, 23]. These absorption bands were broadly spread in the absorption region 464 to 497 cm^{-1} because of Al doping. On calcinations the band was observed shifted to higher wave number and become stronger. This shows that Ag is substituted at site of Zn ions by replacement of Zn ion or at interstitial sites. The absorption bands in the region 439 to 462 cm^{-1} associated vibration was not appearing in pure ZnO structure. The similar assignment has been reported in earlier literature [24]. The calcinations effect was also pronounced and bands were observed shifted to higher frequency region. The vibration band at 667 cm^{-1} in as-prepared Ag doped ZnO assigned to Cd-O-Zn stretching modes which was not appeared in the pure ZnO spectra. This band was found shifted to higher wave number region on increasing the calcinations temperature. The absorption peak present at 1020 cm^{-1} is assigned to C-O-C bending vibrational mode [25]. The absorption band in the range 3425-3533 cm^{-1} was assigned to stretching mode O-H group. This reveals that small amount of water molecules absorbed by the ZnO nanopowder [26, 27].

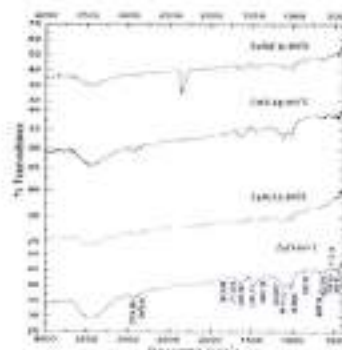


Figure 3 FTIR spectra of ZnO Nanoparticles and Al, Ag and Cd doped ZnO Nanoparticles at 600°C

Vibrational bond	Samples ZnO - 600°C Freq.cm ⁻¹	Samples ZnO Al 600°C Freq.cm ⁻¹	Samples ZnO Ag 600°C Freq.cm ⁻¹	Samples ZnO Cd 600°C Freq.cm ⁻¹
Zn-O	447	464	439	430
Zn-O	470	497	462	452
Zn-O	673	669.32	667	673
Zn-O	923	831	871	834
C=C	1022	1020	1020	1020
C-O	1408	1398.44	1408.08	1440.81
C=O	1600	1581.66	1580.11	1581.68
CO ₂	2369.02	2346.61	2343.50	2359.02
C-H	2861.26	2852.81	2962.76	3026.41
O-H	3425.68	3434.60	3533.71	3425.69

4. UV-Visible Absorption Study for ZnO nanoparticles and Al, Ag and Cd doped ZnO.

The absorption spectrum of pure ZnO, as-prepared and calcined ZnO(Ag) nanopowder was shown in figure 4. All the samples represent significant absorption in ultraviolet and blue region of the absorption spectrum. The absorption intensity was decreased on 5% Ag doping in ZnO. As the calcinations temperature increased the peak height in the blue region decreased and peak shifted to longer wavelength. This concluded that major portion of the absorption spectrum (blue to visible region) can be actively utilized in the solar cell device applications. ZnO sample exhibit a strong absorption peak at about 362 nm. The significant red shift of 362 nm to 378 nm was observed for prepared Al doped ZnO samples. The band gap was calculated using equation $E_g = hc/\lambda$. The estimated band gap and near band edge wavelength is tabulated in table 4. On sintering at 600°C temperature effectively tuned the band gap. Al though the grain size was smaller as compared to pure ZnO nanostructure as-prepared ZnO(Al) exhibit 3.34 eV band gap, which is significantly decreased. Further on increasing temperature of calcinations (600°C) the absorption peak shifted to longer wavelength and band gap was found decreased. The decrease of band gap is related to presence of P-type conductivity of doped semiconductor [29]. The Cd doping in ZnO nanostructure with the impurity level in the energy band gap lead to the formation of P-type in the substance. It was reported that reduction in band gap 3.29 eV led to enhance the efficiency of charge carrier and material is optoelectronically useful [29]. The Cd - doped ZnO nanostructure exhibiting acceptor impurity material may be used in optoelectronics, photoelectrochemical cells, and for biological applications[30, 31].

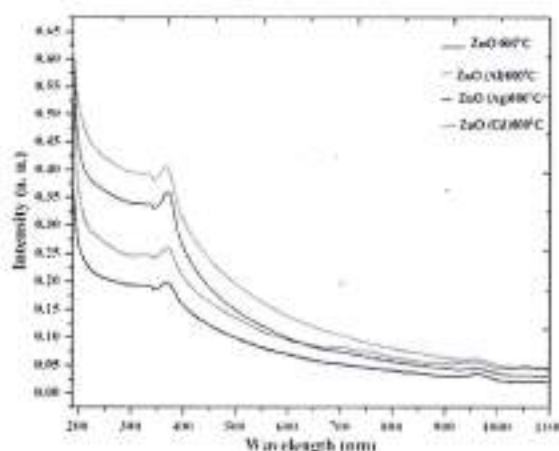


Figure 4. UV absorption spectra of ZnO nanoparticles and Al, Ag and Cd doped ZnO nanoparticles at 600°C.

Table 4. Variation between band gap(Eg) and sintering Temperature		
Samples ZnO-Temperature	Wavelength nm	Bandgap eV
ZnO 600 °C	362	3.44
ZnO (Al) 600°C	373	3.34
ZnO(Ag) 600°C	376	3.31
ZnO(Cd) 600°C	378	3.29

5. Material and Methods

The antimicrobial activity of doped compounds ZnO(Cd), ZnO(Ag) and ZnO(Al) (Calcined at 600°C) are tested in comparison with pure ZnO(calcined at 600°C) nanocompound and Chloramphenicol as known antibiotic agent against *Bacillus Thuringiensis* NCIM2130 and *Pseudomonas cf. monteilii* 9 by slightly modified agar well diffusion method (Perez,et.al,1990) [19] on Muller Hinton agar plates. The *Bacillus thuringiensis* NCIM2130 and *Pseudomonas* were grown separately in Nutrient broth at 37°C for 24 hours on rotary shaker at 200 rpm. Muller Hinton agar plates were inoculated with (100 µl) 24 hours old culture of *Bacillus thuringiensis* NCIM2130 and *Pseudomonas cf.monteilii* 9 spread with sterile glass spreader. The well was made at the centre of inoculated agar plate with a sterile cork borer of 10 mm size. In each well 100µl (1 µgms pure and doped nanocompounds), ZnO(Ag), ZnO(Al), ZnO(Cd) and pure ZnO was poured by using micropipette. The inoculated plates were allowed to stand for 30 minutes for diffusion of poured compounds. Then all these plates are incubated at 37°C for 48 hours. After 48 hours the incubated plates were observed for the activity of tested compounds in terms of zone of inhibition. Zones of inhibition were measured and compared with inhibitory zone of pure ZnO600°C, with Al,Ag and Cd doped ZnO compounds.

5.1 Results and Discussion

Incubated plates of tested doped compounds ZnO(Al), ZnO(Ag), ZnO(Cd), and pure ZnO and Antibiotic Chloramphenicol shows more or less Zones of Inhibition. As per the obtained results, known antibiotic Chloramphenicol shown more antimicrobial activity on both *Bacillus thuringiensis* NCIM2130 and *Pseudomonas cf.monteilii* 9 as compared to pure ZnO compounds. Chloramphenicol has 46 mm zone inhibition against *Bacillus thuringiensis* NCIM2130, 45 mm Zone of inhibition against *Pseudomonas cf.monteilii* 9 respectively.

In comparison with inhibition zone of Chloramphenicol, the inhibition zone of pure ZnO is smaller (16 mm) against *Bacillus thuringiensis* NCIM2130 but pure ZnO and doped ZnO never show antimicrobial activity against *Pseudomonas cf.monteilii* 9. While comparing inhibition zone of pure ZnO with respect to ZnO(Ag), ZnO(Al) and ZnO(Cd) against *Bacillus thuringiensis* NCIM2130 and *Pseudomonas cf.monteilii* 9, doped compounds are more active than pure ZnO. The photo plates of Al doped ZnO, Ag doped ZnO and Cd doped ZnO nanostructures calcined at 600 °C have been shown in figures 5.1, 5.2 and 5.3 respectively. The diameters of inhibition zones for Al doped ZnO, Ag doped ZnO and Cd doped ZnO are tabulated in table 5. The zone of inhibition for doped ZnO(Cd) is 39 mm, for ZnO(Ag) is 32 mm and for ZnO(Al) is 26 mm against *Bacillus thuringiensis* NCIM2130 where as for ZnO(Cd) is 0 mm, for ZnO(Ag) is 0 mm, and for ZnO (Al) is 0 mm zone of inhibition against *Pseudomonas cf.monteilii* 9 have been observed.

On the basis of obtained results pure ZnO has antimicrobial activity but modified doped ZnO compounds are more active than pure ZnO compound against *Bacillus thuringiensis* NCIM2130 where as pure ZnO and doped ZnO(Cd), ZnO(Ag) and ZnO(Al) compounds has no effect or less active against *Pseudomonas cf.monteilii* 9 have been observed. The effect of doping and calcinations of the material are essential factors for increase the antibacterial activity. The literature on ZnO reported that Ag doped

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ZnO and Al doped ZnO materials are bio safe material [3, 7, 10, 11]. The effect of doping decreased the grain size of nanoparticles for as-prepared doped ZnO nanostructure. The grain size of Al doped ZnO was 44.18 nm, that of Ag doped ZnO was 46.36 nm and of Cd doped ZnO was 41.54 nm. The particle size estimated for pure ZnO is about 48.73 nm. The grain size is one of the important factor which helps to activate the material at particular site of the bacterial cell and prevent the growth. On calcinations the grain size was slightly increased due to aggregation of the particles however the microstructure of surface morphology of the ZnO and doped ZnO nano structures was modified which was described. The change in particle size and modification of surface morphology leads to increase the antibacterial activity of ZnO(Al), ZnO (Ag) and ZnO (Cd) nanostructures. The summarizing the figures 5.1, 5.2 and 5.3, table number 5 it is cleared that ZnO (Ag) and ZnO (Cd) nanostructures are very effectively useful for antibacterial activity against *Bacillus thuringiensis* NCIM2130. Among pure ZnO, Al doped ZnO and Ag doped ZnO the Cd doped ZnO calcined at 600 °C showing good results. Therefore ZnO(Cd) nano sample can be used as antibiotic inhibitor against *Bacillus thuringiensis* NCIM2130. To increase the activity and stability the doping percentage of Cd should have to be increased.

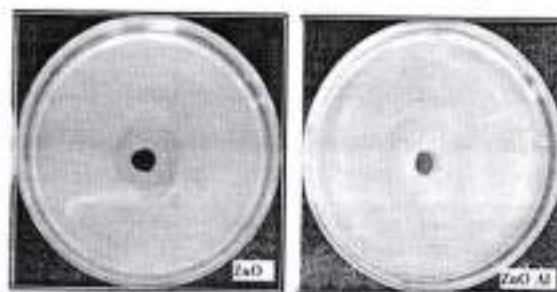


Figure 5.1 Photo plate shows Inhibition zone of *Bacillus Thuringiensis* NCIM 2130 ZnO(Al) in comparison with ZnO nanoparticles at 600°C.

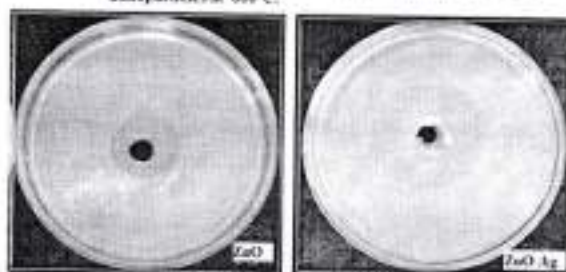


Figure 5.2 Photo plate shows Inhibition zone of *Bacillus Thuringiensis* NCIM 2130 ZnO(Ag) in comparison with ZnO nanoparticles at 600°C.

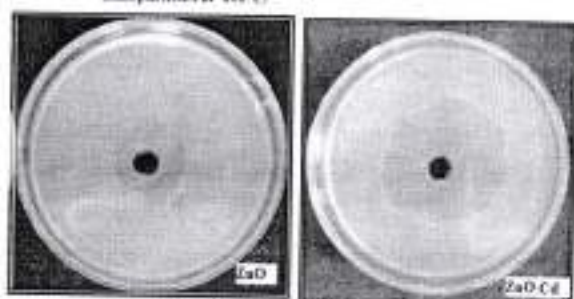


Figure 5.3 Photo plate shows Inhibition zone of *Bacillus Thuringiensis* NCIM 2130 ZnO(Cd) in comparison with ZnO nanoparticles at 600°C.

Table 5. Inhibition Zone (mm) Zones produced by ZnO nano particles at 600 °C calcinations Temperatures								
Bacterial Culture	Inhibition Zone (mm)							
	ZnO 600°C	Cd	ZnO 600°C	Ag	ZnO 600°C	Al	ZnO 600°C	Pure
<i>Bacillus thuringiensis</i> NCIM2130	39		32		26		16	

<i>Pseudomonas cf. monteilii</i> 9	0	0	0	0
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Conclusion:

The prepared novel materials, Pure ZnO600°C, Al doped ZnO, Ag doped ZnO and Cd doped ZnO 600°C were tested against *Bacillus thuringiensis* NCIM2130 and *Pseudomonas cf. monteilii* 9 for antibacterial activity. The entire samples show more or less inhibition activity against *Bacillus thuringiensis* NCIM2130 however no significant effect on *Pseudomonas cf. monteilii* 9 culture. The effect of calcinations temperature enhances the inhibition activity against *Bacillus thuringiensis* NCIM2130. In comparative study of pure and doped ZnO nanoparticles, Al doped ZnO, Ag and Cd doped ZnO nanostructures calcinated at 600°C shows good results against *Bacillus thuringiensis* NCIM2130. However among pure ZnO, Al doped ZnO and Ag doped ZnO the Cd doped ZnO calcined at 600°C showing versatile results. Therefore ZnO(Cd) nano sample can be used as antibiotic inhibitor against *Bacillus thuringiensis* NCIM2130.

The grain size is one of the important factor which helps to activate the material at particular site of the bacterial cell and prevent the growth. The effect of calcinations improved physical properties such as crystallinity, band gap, surface morphology of the nanoparticles that change is responsible to activate the antimicrobial activity of doped ZnO nanoparticles. The doping of different element in ZnO shows varying antibacterial activity against *Bacillus thuringiensis* NCIM2130. In the present study Cd doped ZnO calcined at 600 °C temperature is significantly effective as microbial inhibitor against *Bacillus thuringiensis* NCIM2130.

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