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SYNTHESIS OF Zn SUBSTITUTED $MnAl_2O_4$ (ZMA) BY SIMPLE SOL GEL AUTO-COMBUSTION METHOD: CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY

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ABSTRACT

The sol-gel auto-combustion process is a known wet chemical method applied for the synthesis of composites, especially Mixed Transition Metal Oxides (MTMOs). Herein, we report the sol-gel auto combustion method for the preparation of Zn-substituted $MnAl_2O_4$ (% of Zn varies from 0%, 25%, 50%, 75% and 100%) using respective metal nitrates as a precursor. The as-synthesised materials were characterised by using X-Ray Diffraction (XRD), Field Emission-Scanning Electron Microscopy (FE-SEM), Energy-dispersive X-ray spectroscopy (EDS), Fourier Transform-Infrared spectroscopy (FT-IR), UV–Visible spectroscopy (UV–Vis.) and Thermal Analysis (TGA). Moreover, the antimicrobial properties of the synthesised compositions of ZMA (0%, 25%, 50%, 75% and 100%) against both lab isolated Gram-positive (*S. aureus*) and Gram-negative (*E. coli, P. aeruginosa, Salmonella spp.*), were tested by the agar well diffusion method. The study clearly reveals that ZMA (50%) composition has a strong antibacterial potential.

Keywords: Zn Substituted $MnAl_2O_4$, ZMA, Sol-gel Auto Combustion, Mixed Transition Metal Oxides (MTMOs), Antimicrobial Activity, *Salmonella spp., E. coli, P. aeruginosa* and *S. aureus*

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INTRODUCTION

Spinel structured MTMOs belonging to the Fd3m space group are known as promising materials due to their excellent applications in a various fields such as catalysis, ceramics, lasers, fluorescent materials, sensing materials, dielectric materials and biological materials.[1-7] Among those MTMOs, the mixed transition metal oxides containing aluminates have attracted extensive attention due to their different properties like crystal structure, high chemical and thermal stability, wide range of selectivity, high mechanical strength, low surface acidity and excellent optical and dielectric properties.[8-10] The MTMOs have the general chemical formula expressed as AB_2O_4, where A represents a bivalent metal cation, occupies the tetrahedral position, and the tetrahedral void is occupied by four oxygen ions, while B represents a trivalent cation, occupies the octahedral position, and the octahedral void is occupied by six oxygen ions.[11-16] While synthesising MTMOs, by choosing a sol-gel auto combustion wet chemical method, the researcher can get the choice of doping a desired metal ion and the method can allow reduction in size of the material with close packing.

Obviously, the sol-gel auto combustion method can enhance the crystal structure and band structure of the MTMOs.[17-20] Here, doping of a suitable metal provides the hole or electron carrier, which ultimately controls the electrical, optical, magnetic and antimicrobial properties of new synthesized MTMOs.[21-24] Many investigations have been reported for the preparation and characterisation of transition metal ion-

doped zinc aluminate. The efforts have also been made to enhance the luminescent properties by doping rare-earth metal ions in the spinel-structured lattice of zinc aluminate. This predicts that the physical properties of spinel structure metal-oxide strongly depend upon the synthetic process, doping elements and doping amount/per cent.[25-29] Furthermore, many reports were encountered, especially over manganese oxides such as MnO, MnO_2, Mn_2O_3 and Mn_3O_4, and it remarkably attracts the attention of the researcher in the applications of wastewater treatment, enhanced catalysis, selective sensors, supercapacitors, and as an anode material in rechargeable lithium-ion batteries for their low cost and high capacity with environmental benignity. Specifically, MnO nanoparticles have biological applications such as drug delivery and imaging.[7]

However, there are scant reports on Mn-doped $ZnAl_2O_4$ for their structural properties and their applications.[30-34] But still, researchers have to fill the gap of antimicrobial investigation for the influence of Mn-substituted $ZnAl_2O_4$, where the amount of substitution varies from 0% to 100%. Furthermore, the synthesis of Mn-substituted $ZnAl_2O_4$ by the sol-gel auto-combustion method, spectroscopic characterisation, electrical properties, magnetic properties, and its experimental applications, such as catalysis and antimicrobial activity, have not been reported so far. In continuation with our previous work,[51-58] Zn-substituted $MnAl_2O_4$ (ZMA) is synthesized by sol gel auto-combustion method followed by calcination treatment.

The effects of Zn-substitution content on the crystal structure, morphology and composition were characterised by X-ray diffraction (XRD), Field Emission-Scanning Electron Microscopy (FE-SEM), Energy Dispersive X-ray spectroscopy (EDX), Fourier Transform-Infrared spectroscopy (FT-IR), Ultraviolet-Visible (UV-Vis) spectroscopy and Thermogravimetric Analysis (TGA). Recently, *Gadkov et al.*[35] and *Raghunath et al.* [36] took over special emphasis on metal oxides as a versatile antimicrobial agent against *E. coli, S. aureus and P. aeruginosa.*[37-50] Some reports predict the enhancement in antibacterial activity by the addition of Zn and Mn-containing mixed metal oxides.[59-62] Hence, the further work focused on the performance of Zn-substituted $MnAl_2O_4$ (ZMA) mixed metal oxide for antimicrobial activity against *E. coli, S. aureus and P. aeruginosa and Salmonella spp.*

EXPERIMENTAL

Material and Methods

All the metal salt precursors such as a Zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$, MW 297.48 gm), Manganese nitrate ($Mn(NO_3)_2 \cdot 4H_2O$, MW 251.01 gm), Aluminium nitrate ($Al(NO_3)_3 \cdot 9H_2O$, MW 375.13 gm), Chelating agent as a Citric acid ($C_6H_8O_7.2H_2O$, MW 210.14 gm) and 1:1 Ammonia (NH_4OH) for neutralization were procured as an analytical reagent grade (A. R. grade) and were used without any further purification. De-ionised water (DI)) was used for dissolving the precursors, such as metal nitrates and citric acid.

General Procedure for the Synthesis of ZMA

In this work, five different compositions of ZMA (% of Zn varies from 0%, 25%, 50%, 75% and 100%) were synthesised by the sol-gel auto-combustion method. Citric acid ($C_6H_8O_7 \cdot 2H_2O$) was used as a chelating agent. For the typical synthetic procedure of each composition of ZMA, the calculated amount of zinc nitrate and manganese nitrate precursors was dissolved in a minimum amount of DI water. Similarly, an equimolar amount of citric acid was also dissolved in DI water. Dissolved nitrate salt and citric acid solutions were mixed in a borosilicate glass beaker. Then, with constant stirring, an appropriate amount of 1:1 NH_4OH solution was added until complete neutralisation of the solution (pH = 7 to 7.5). The pH was monitored on a pH meter using a glass electrode.

The resulting neutralised solution was evaporated on a hot plate with constant stirring at about 100 ℃ and further continued rigorous heating until the solution turned into a viscous gel, followed by fine powder. During the rigorous heating, auto-combustion was observed. The powder was ground to its fineness in a mortar and pestle to achieve homogeneity. The fine powder was collected in a silica crucible and subjected to pre-sintering at a temperature of 600 ℃ for 6 h and finally sintered at 1000 ℃ for 10 h in a muffle furnace. Similarly, each fine crystalline material of ZMA with a different percentage composition was synthesised and further characterised.

Characterization

The X-ray diffraction (XRD) analysis was carried out using AXS D8 Advance, Bruker Ltd., Germany (Cu, λ= 1.5406 Å). Fourier Transform Infrared spectrum (FT-IR) was recorded on a Bruker ALPHA 100508 spectrometer. Thermo-Gravimetric Analysis (TGA) was done using SDT Q600, TA Instruments. The microscopic surface and compositional analysis were recorded by using Field Emission-Scanning Electron Microscopy (FE-SEM) of make JED-2200, JEOL, Tokyo, Japan. The FE-SEM sample was fixed onto a stub with carbon-built dual-sided adhesive tape and then sputter-coated with nearly 10 nm of platinum. For imaging, the sample is focused perpendicular to the inward electron beam. FE-SEM pictures were captured using an acceleration voltage of 20 kV and a working distance of 5 mm over several magnifications. Moreover, the antimicrobial properties of the different compositions of ZMA against both lab isolated Gram-positive (*S. aureus*) and Gram-negative (*E. coli, P. aeruginosa, Salmonella sp.*), were tested by the agar well diffusion method.

RESULTS AND DISCUSSION

TGA/DTA/DSC Analysis

Figure-1 shows the thermal stability of the synthesised ZMA (50%) material, which was performed under N_2 atmosphere at a heating rate of 10 ℃/min. Figure-1a shows the TGA graph of Weight loss (%) versus Temperature (^{0}C). The weight loss of 9.9% in the range of 50 to 130 ^{0}C could be observed due to the evaporation of physically absorbed water molecules. Subsequent weight loss of 13.14% in the vicinity of 130 to 380 ^{0}C is attributed to the thermal decomposition of organic molecules existing in the dried precursor. Finally, steady weight loss in the temperature range of up to 1100 ^{0}C is probably due to the complete desorption of nitrate ions.[63] Based on the thermogram, sintering was done at 1000 ^{0}C to obtain fine crystalline material. Figure-1b, the graph of Heat flow (W/g) versus Temperature (^{0}C) and Fig.-1c, the graph of Temperature difference (^{0}C/mg) versus Temperature (^{0}C), also support the data.

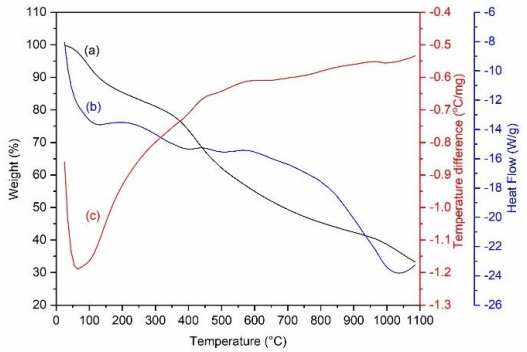


Fig.-1: Thermograms of ZMA (50%) composite a) Weight loss Vs Temperature (TGA) b) Heat flow Vs Temperature (DSC) c) Temperature difference Vs Temperature (DTA)

XRD Analysis

The phase and purity of all the synthesised compositions of the ZMA were identified by XRD studies. Figure-2 shows their obtained diffraction patterns. Figure-2(a-d) shows the diffraction peak positions at 2θ values 31.31°, 36.89°, 45.29°, 49.10°, 55.67°, 59.39°, 65.31°, 74.18° and 77.43° corresponds to respective crystal planes such as (220), (311), (200), (400), (331), (422), (511), (440), (620) and (533). This is in good agreement with the JCPDS card #29-0880.[64.] This clearly indicates the presence of $MnAl_2O_4$ in the ZMA (0%, 25%, 50% and 75%). Similarly, Fig.-2(b-e) shows the diffraction peak positions at 2θ values at 31.13°, 36.70°, 44.60°, 48.94°, 55.46°, 59.17°, 65.04°, 73.89° and 77.04° corresponds to respective crystal planes such as (220), (311), (200), (400), (331), (422), (511), (440), (620) and (533). This is in good agreement with the JCPDS card #05-0669.[65.] This clearly indicates the presence of $ZnAl_2O_4$ in the ZMA (25%, 50%, 75% and 100%). ZMA (0%) and ZMA (100%) both have a cubic space lattice (Fd3m) shape with a lattice parameter (a=b=c) of 8.0950°A and 8.099 °A, respectively. Thus, as can be seen in Fig.-2(b, c, and d), the XRD pattern of ZMA (25%, 50%, 75%), all of the XRD peaks are sharp, but some of them are merged and seem to be a sharp doublet having close 2θ angles. This sharp

doublet indicates $ZnMnAl_2O_4$ is a composite mixture of $ZnAl_2O_4{\cdot}MnAl_2O_4$ mixed metal oxide.[66] The XRD reflections observed in Fig.-2 of all the as-synthesised compositions of ZMA composites show a more intense 311 peak, indicating a spinel structure, and perfectly coincide with the reported diffraction data.[66] Ultimately, comparison of XRD spectrographs observed in Fig.-2(a-e) shows a left shift in 2θ values, indicating that increasing doping concentration of Zn^{2+} ions is gradually and effectively added into the $MnAl_2O_4$ lattice.

FT-IR Analysis

FTIR spectroscopy is a unique technique to detect the bonding of mixed transition metal oxides (MTMOs). Fig.-3(a-e) shows FTIR spectra of ZMA (0%, 25%, 50%, 75%, 100%). Each spectrum was recorded in the range of 400-4000 cm^{-1}. Figure-3(a-d), i.e. FTIR spectrum of each sample of ZMA (25%, 50%, 75%, 100%) except ZMA (0%), particularly shows absorption bands around 3417-3421 cm^{-1}, 1635-1638 cm^{-1}, 801-809 cm^{-1}, 658-666 cm^{-1}, 555-557 cm^{-1} and 479-494 cm^{-1}. The absorption band at 658-666 cm^{-1} corresponds to the symmetric stretching vibration of the Al-O bond (in AlO_6 group), while the absorption band at 555-557 cm^{-1} is attributed to the symmetric bending vibration of the Al-O bond (in AlO_6 group). These bands can be assigned to the characteristic of the spinel structure.[76] The absorption band at 479-494 cm^{-1} attributes to Zn-O stretching[66,74-77] and 801-809 cm^{-1} attributes Zn-O-Al stretching.[74] In addition, the wide absorption band at 3417-3421 cm^{-1} can be attributed to the stretching vibration of the H-O-H group, and the absorption near about 1635-1638 cm^{-1} can be assigned to the bending vibration of the O-H group, both of which are related to water molecules on the surface of the sample.[66,67,70-73,75,78-80] On the other hand, FTIR spectra of ZMA (0%) show characteristic absorption at 443 cm^{-1} (Mn-O),[66-73] 594 cm^{-1}(Al-O),[66,74,75,77,78] 640 cm^{-1} (Mn-O-Mn),[66] 799 cm^{-1} (Mn-O-Al),[66] 1636(H-O-H),[66,67,70-73,78-80] 3419(O-H).[66,67,70,75,79]

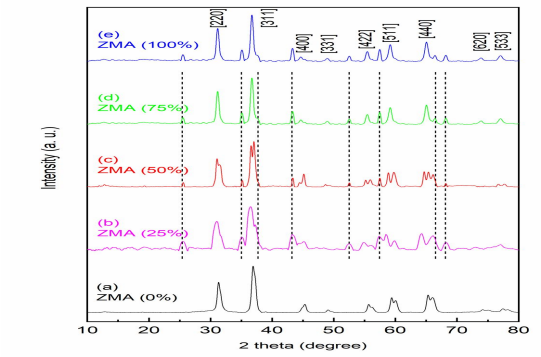


Fig.-2: X-ray Diffraction (XRD) Patterns of Synthesised (a) ZMA (0%), (b) ZMA (25%), (c) ZMA (50%), (d) ZMA (75%), (e) ZMA (100%)

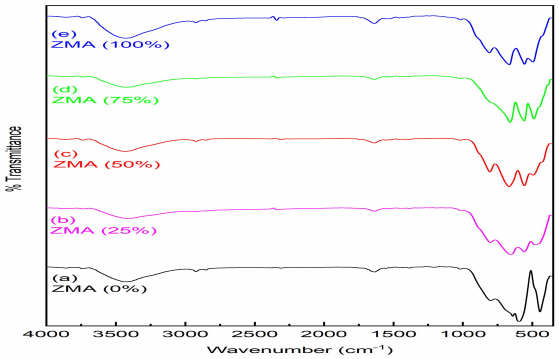
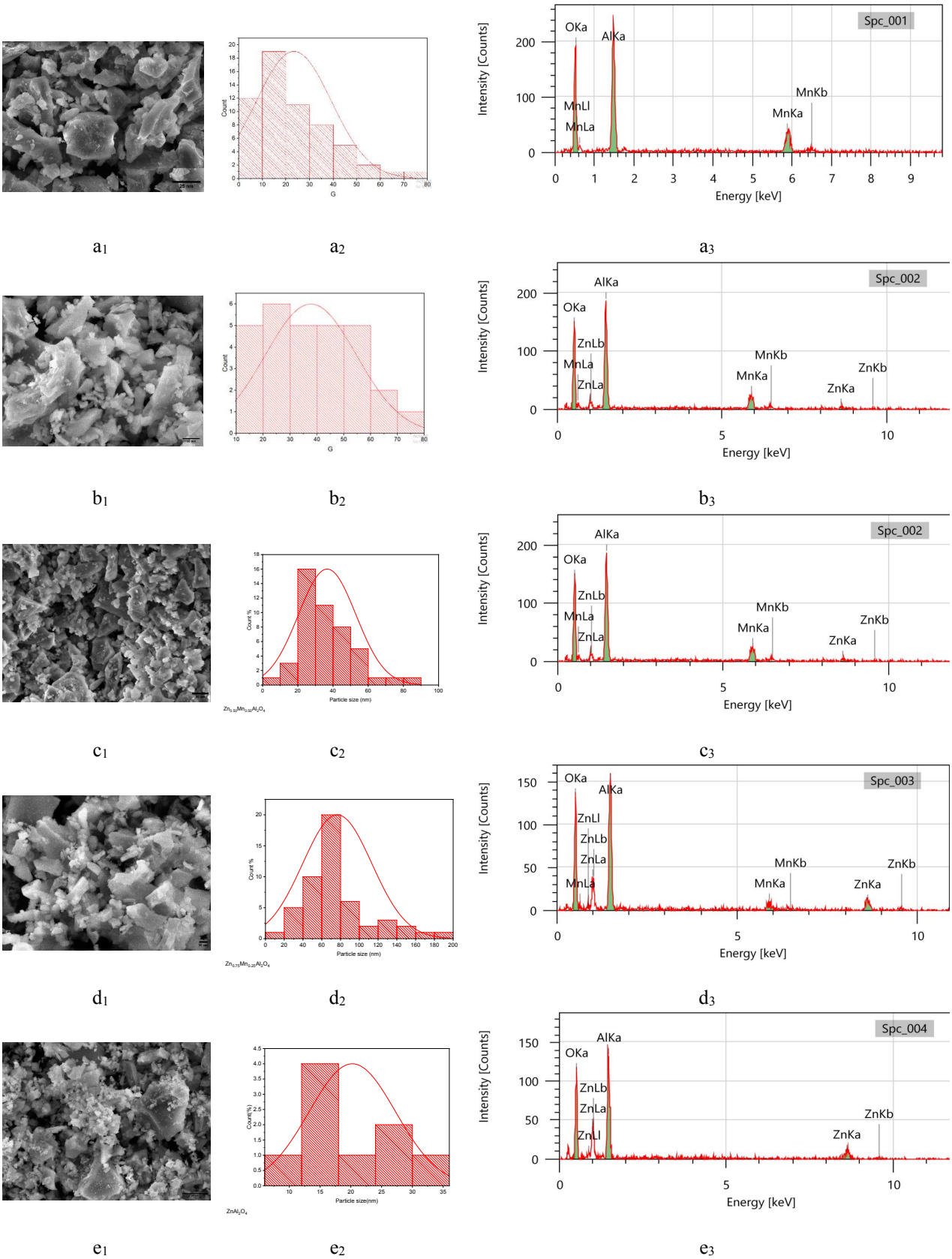


Fig.-3: FT-IR Spectrum of as-synthesised (a) ZMA (0%), (b) ZMA (25%), (c) ZMA (50%), (d) ZMA (75%), (e) ZMA (100%)

FESEM - EDS Analysis

To prove that, Zn^{2+} successfully replaces Mn^{2+} and enters the $MnAl_2O_4$ matrix. The samples were analysed by FESEM and EDS. The FESEM images shown in Fig.-4 predict randomly distributed flakes like surface morphology in all compositions of ZMA (0%, 25%, 50%, 75%, 100%). The histogram (Fig.-4a 2 to $4e_2$) shows that grains or agglomerated particles are found with a size range between 50nm and 100nm. These grain size values obtained from the histogram are in good agreement with the estimated values from the XRD pattern. EDS analysis of all the compositions of ZMA composites was carried out, and the elemental mapping was performed to determine the distribution of composition in the matrix. Figure-4a 3 to $4e_3$ shows the EDS mapping spectrum of the synthesised materials. In the sample of ZMA (0%) composite, only Mn, Al and O atomic per cent values were found (Fig.-$4a_3$), and in the sample of ZMA (100%) composite, only Zn, Al and O atomic per cent values were found (Fig.-$4e_3$). On the other hand, ZMA (25%, 50%, 75%) shows peaks of Zn, Mn, Al and O (Fig. $4b_3$-d_3). The average values of atomic per cent of constituent atoms with standard deviation are presented in Table-1. The Zn atom percentage increases with increasing Zn doping concentration, and the atom percentage of Zn directly delineates the original doping percentage and further illustrates the increasing trend.



a_1 a_2 a_3

b_1 b_2 b_3

c_1 c_2 c_3

d_1 d_2 d_3

e_1 e_2 e_3

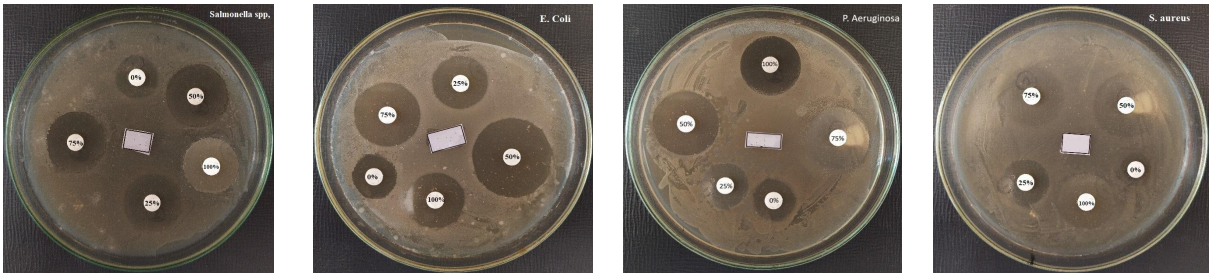
Fig.-4: FESEM, Histogram and EDS Analysis of Synthesised ZMA 0% (a_{1-3}), ZMA 25% (b_{1-3}), ZMA 50% (c_{1-3}), ZMA 75% (d_{1-3}), ZMA 100% (e_{1-3})

Table-1: Elemental Composition of ZMA by EDS Analysis

Compositions	Atomic percent (%)			
	Zn	Mn	Al	O
ZMA (0%)	Not detected	11.92 ± 0.57	29.38 ± 1.01	58.70 ± 1.78
ZMA (25%)	3.07 ± 0.43	11.56 ± 0.55	28.18 ± 1.14	57.19 ± 2.02
ZMA (50%)	7.05 ± 0.41	6.25 ± 1.46	29.96 ± 1.39	56.74 ± 1.73
ZMA (75%)	11.00 ± 0.80	3.29 ± 0.30	28.37 ± 1.25	57.34 ± 2.13
ZMA (100%)	13.38 ± 0.90	Not detected	28.04 ± 1.38	58.68 ± 2.41

Antimicrobial Activity of ZMA (0%, 25%, 50%, 75%, 100%)

ZMA composites with composition 0%, 25%, 50%, 75%, 100% were synthesised by the sol-gel auto combustion method. The synthesised materials were characterised for their structural and compositional study. Further, all the compositions of ZMA were studied to check their potential as anti-bacterial agents. The anti-bacterial activities of ZMA against laboratory isolates, including both Gram-positive (*S. aureus*) and Gram-negative (*E. coli, P. aeruginosa, Salmonella spp.*), were tested by the agar well diffusion method. The results are shown in Fig.-5 and in Table-2. The anti-bacterial activity of the ZMA was evaluated by using the agar well diffusion assay method. The test organisms, *E. coli, S. aureus, P. aeruginosa,* and *Salmonella spp*, were grown in nutrient broth overnight to attain colony-forming units (CFU) of 10^6 cells per ml. 100 mL of each bacterial culture was then spread and inoculated on the nutrient agar plates. The discs of 5 mm diameter, soaked with different compositions of ZMA in DMSO (Dimethyl sulfoxide), were placed at the centre of the nutrient agar plate. Then the plates were incubated at 37 ℃ for 24 hours. The antibacterial activity was assessed by determining the diameter of the zone of inhibition against the bacteria. In this study, a clear zone was observed with different diameters of zone of inhibition at different concentrations, such as 0%, 25%, 50%, 75% and 100%. The results are presented in Table-2 and Fig.-6. The inhibitory zone was found to be highest for the ZMA (50%) sample**.**



Salmonella spp. *E. coli* *P. aeruginosa* *S. aureus*

Fig.-5: Photographs of the Inhibition Zone for Each Bacterium Under Consideration

Table-2: Anti-bacterial Activity of the ZMA Material

Composition	Inhibition zone (mm).			
	Salmonella spp.	*E. coli*	*P. aeruginosa*	*S. aureus*
ZMA (0%)	8	9	9	5
ZMA (25%)	11	12	10	9
ZMA (50%)	19	20	18	14
ZMA (75%)	16	18	16	12
ZMA (100%)	13	15	13	11

Note: ZMA material is dissolved in DMSO (Concentration 1000 μg/ml)

Results further predict that the zone of inhibition was highest for the composition ZMA with a concentration of 50% against gram-negative bacteria *Salmonella* spp (19 mm), *E. coli* (20 mm) and *P. aeruginosa* (18 mm) when compared to the gram-positive bacterium *S. aureus* (14 mm). This is due to the thicker peptidoglycan layer of the cell wall of gram-positive bacteria, which makes them less sensitive than gram-negative bacteria. The maximum inhibition was observed against *E. coli,* and less against *S. aureus*, as observed in Fig.-6. The reasons for the antibacterial activity of the ZMA composites are the large surface area of the composites, so that they can tightly bind to the surface of the bacterial cells to

disrupt the membrane, leading to the leakage of intracellular components and killing the bacterial cells. Observed toxicity may be attributed to the electrostatic interaction between ZMA mixed transition metal oxide particles and the bacterial cell membrane. Generation of reactive oxygen species (ROS) like hydroxyl ions, peroxides, superoxide and hydrogen peroxide may be the main reason for the toxicity of ZMA mixed transition metal oxide particles. The lethal agent hydrogen peroxide, which is actually produced from hydroxyl ions and peroxide free radicle could damage the cell by complete destruction of the membrane of bacterial cells.[82] The MIC is the lowest concentration of the antibacterial agent that inhibits the visible growth of bacteria. The MIC is used to evaluate the antimicrobial efficacy of different compounds. In the present study, MIC of the ZMA (50%) was evaluated by the modified dilution method as described by *Tayel et al*.[81] In this method 0.1 ml of 24-hour-old bacterial cultures (~10^6 CFU/mL) were mixed in test tube with 0.1 mL of a ZMA (50%) suspension (1000 ug/ml in DMSO) along with 10 ml of broth media. The tubes were then incubated overnight, and MIC was determined as the minimal concentration of ZMA (50%) that completely inhibited bacterial growth as observed by measuring optical density at 540nm. Table-3 and Fig.-7 show Minimum Inhibitory Concentration (MIC) values for the ZMA (50%) determined by observing the growth in terms of optical density at 540 nm. Turbidity analysis displayed that 600 and 700 µg/ml concentration of the ZMA (50%) inhibited the growth of *S. aureus* and *E. coli,* and they were considered as MIC values, respectively, while for *Salmonella spp* and *P. aeruginosa,* MIC was found to be 800 & 900 µg/ml. For different mixed transition metal oxides, similar values of MIC were made by *Matai et al.*[83] and *Paul et al.*[84]; however, greater MIC values were reported by *Lu. Et al.*[85] MIC was further confirmed by inoculation of fresh bacterial cultures again at inhibitory concentrations and observed no growth for the ZMA (50%) with 600 and 700 µg/ml for *S. aureus* and *E. coli,* respectively, and at 800 & 900 µg/ml for *Salmonella* spp and *P. aeruginosa,* respectively, which were considered as MKC value. This study clearly proposes a strong antibacterial potential of ZMA (50%).

Table-3: Minimum Inhibitory Concentration (MIC) of ZMA (50%)

100	0.69	0.47	0.85	0.38
200	0.63	0.36	0.72	0.23
300	0.52	0.21	0.60	0.14
400	0.47	0.18	0.48	0.09
500	0.31	0.10	0.36	0.06
600	0.14	0.03	0.24	0.0
700	0.02	0.0	0.11	0.0
800	0.0	0.0	0.01	0.0
900	0.0	0.0	0.0	0.0
1000	0.0	0.0	0.0	0.0

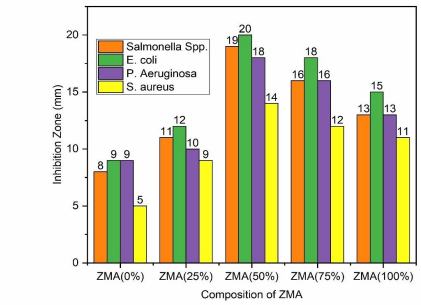


Fig.-6: Anti-bacterial Activity of the ZMA (0%, 25%, 50%, 75%, 100%,)

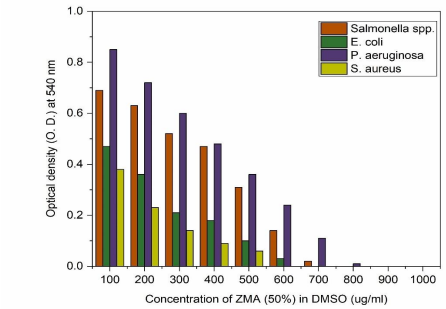


Fig.-7: Minimum Inhibition Concentration of ZMA (50%)

CONCLUSION

As is clear from the result, ZMA (50%) shows enhanced antimicrobial activity. Hence, substitution of the 50% concentration of Zn in the $MnAl_2O_4$ lattice shows a profound effect on antimicrobial activity against

a variety of bacteria. The mixed metal oxide of Zn, Mn, and Al, i.e. $ZnMnAl_2O_4$, shows maximum antimicrobic activity than individual pure analogues such as $MnAl_2O_4$ and $ZnAl_2O_4$. Still, metal ion doping such as Fe, Ni, Mg and other transition metals needs to be studied further.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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