



Synthesis and Characterization of Iron-Zinc-Aluminum Mixed Metallic Oxide from Metal Scraps Focusing on Its Application in Antifungal Paints

Abstract: The use of sustainable multifunctional pigments obtained from secondary raw materials has been identified as an effective strategy to improve the performance of environmentally friendly protective coatings. In this study, Fe-Zn-Al oxide nanoparticles were obtained from metal waste electrochemically and used as an antifungal pigment in aqueous paints. Scrap metal, galvanized steel, aluminum and zinc were dissolved and electrochemically filtered, calcined at 700 °C and ball milled to obtain mixed metal oxide nanoparticles. These pigments were analyzed by XRD, FESEM-EDS, FTIR and UV-Visible spectroscopy. XRD results demonstrated that Fe-Zn-Al mixed metal oxides with hematite structure were formed with highly crystalline nanoparticles, while FESEM-EDS results proved uniform distribution of Fe, Zn, Al and O elements. Formation of metal-oxygen bonds was confirmed by FTIR spectroscopy whereas UV-Vis absorption spectra were found to be broad for mixed metal oxides with defects. Antifungal activity was evaluated against *Penicillium*, *Aspergillus niger*, *Aspergillus fumigatus* and *Rhizopus* sp. using diffusion techniques in agar wells and gravimetric reduction of biomass. It is clear that these nanoparticles had antifungal activity in a dose-dependent manner with greater activity against *Penicillium* and *Rhizopus* sp. These findings suggest that electrochemically synthesized Fe-Zn-Al mixed metal oxide nanoparticles derived from waste material are suitable for use as environmental friendly multi-functional pigments for antifungal water-based paints.

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1. Introduction

Multifunctional inorganic pigments have gained a lot of interest because of their high chemical stability, durability and environmentally friendly properties. [1] The optical, thermal and protective properties of mixed metal oxide pigments are better than other pigments, so they are more suitable to be used in high value coating applications. Chemical stability, corrosion protection, color stability and relatively nontoxic properties make Fe₂O₃, ZnO, and Al₂O₃ good inorganic pigments for protective coating applications [2-6] The iron, zinc and aluminum oxides can then be used to develop a combined oxide pigment system that will create synergistic effects resulting in improved pigment and paint performance. But the microbial contamination of painted surface is an important issue nowadays and development of antimicrobial coatings is under consideration. [7], [8] Fungi can alter the aesthetic properties of painted surfaces, damage paints and can also be harmful to health in humid indoor

environments. [9], [10] The inclusion of such inorganic functional pigments in the paint formula can be a solution to acquire fungicidal properties while maintaining adequate mechanical and aesthetic properties. [11-13] Iron-zinc-aluminum oxides are the most promising candidates due to the structural stability, morphological control of particles, and potentially antifungal properties due to the surface chemistry of the metal oxides. The electrochemical route employed in this study minimizes the use of additional chemical reagents, operates under relatively mild conditions, and generates hydrogen at the cathode as a co-product. [14-16] Therefore, the objectives of this research are to synthesize Fe-Zn-Al oxide NPs by recycling metal waste by electrochemical technique and to characterize the physical and chemical properties of the NPs; and to evaluate the antifungal activity of the NPs and recommend them as durable multifunctional pigments for water-based paints.

2. Materials and Method

2.1. Materials

Galvanized iron (GI) scrap and zinc metal were used as the anode and cathode, respectively, for the electrochemical synthesis of the Fe-Zn mixed oxide. When using compositions containing aluminum, scrap aluminum was used as the anode and cathode. [17] Sodium chloride (NaCl, analytical grade, 99.9%) and deionized water were used to prepare the electrolyte. Potato Dextrose Agar (PDA) and *Penicillium* spp., *Aspergillus niger*, *Rhizopus* sp. and *Aspergillus fumigatus* spore suspension were used for the antifungal evaluation. All the experimental procedures were repeated using laboratory glassware and filters. The sample treatment, drying, calcination and mixing were

carried out by drying oven, muffle furnace, ball mill and 25 μm sieves.

2.2. Electrochemical Synthesis of Fe-Zn mixed oxide and Fe-Zn-Al mixed Oxide

Wastes in metal form were first collected, sorted and cleaned mechanically from rust, coatings, oils and impurities that were sticking to the metal. The pieces were washed, degreased, surface activated and then subjected to electrochemical processing. An amount of 21 g of sodium chloride was dissolved in 150 mL of deionized water to produce the electrolyte. Purified galvanized iron (GI) and zinc scraps were used as sacrificial anodes with galvanized iron (GI) as the cathode for the synthesis of Fe-Zn mixed oxide. For surface cleaning, electrolysis was first carried out at 30 V and about 5 A for 4 h, and then under controlled conditions at progressively lowered voltages (from 30 to 5 V) and currents (from about 5 to 3 A) for various amounts of time. The precipitate formed was filtered on Whatman No. 42 paper, dried at 110 °C, then calcined at 700 °C and ball-milled for 3 h to obtain the Fe-Zn mixed oxide powder.

The Fe-Zn oxide was prepared by the above procedure while the aluminum oxide was separately produced by electrolysis of purified aluminum can scrap in the same NaCl electrolyte with aluminum as anode and cathode under the following conditions: current 5 A and voltage 30 V for 3.5 h. The recovered solids containing aluminum was filtered, dried at 110 °C, calcined at 800 °C and ball-milled 1 h. Fe-Zn mixed oxide and Al_2O_3 powders were prepared by the above method and homogenized by ball milling in the ratio of 8:2 by mass. Fe-Zn-Al mixed Oxide was then obtained by calcinating the mixture in graphite crucibles for 4 h at 700 °C, to encourage the formation of mixed-oxide phases.

The developed system was evaluated by two independent evaluators, comprising one evaluator from the Department of Chemistry and one evaluator from the Department of

Microbiology. Their assessments were used to evaluate the system's performance and suitability for the intended application.

Table 1. Iron-Zinc-Aluminum Oxide Formulation

Composition Group	Oxide Ratios	Calcination Temp. °C	Calcination Time, hr
Fe ₂ O ₃ -ZnO: Al ₂ O ₃	8.: 2	700.	4.

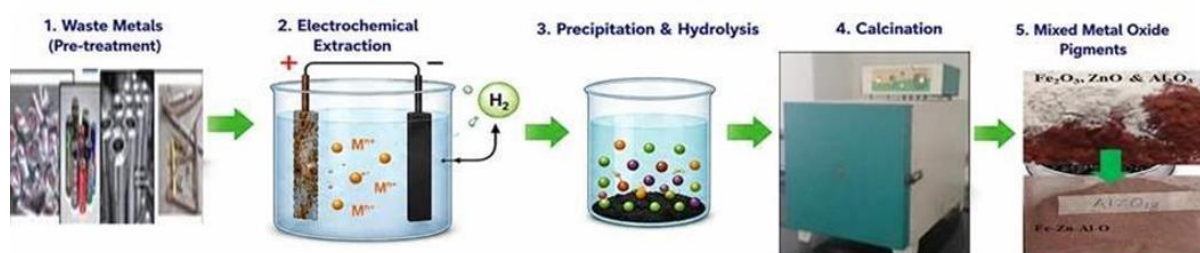


Figure 1 Electrochemical Conversion of Iron, Zinc and Aluminum scraps in to Fe-Zn-Al Mixed Metal Oxide Pigments

2.3. Physicochemical Characterization of Iron-Zinc-Aluminum Oxide NPs

The synthesized Fe-Zn-Al Oxide NPs were well characterized to investigate their morphological, elemental, structural, surface chemical and optical properties. The FESEM, EDS, XRD and FTIR were used to analyze the morphology, elemental analysis, crystalline structure, crystallite size and chemical bonding and surface chemistry, while optical absorbance was analyzed using UV-Visible spectroscopy. This combination of different techniques made it possible to obtain a detailed overview of the physical and chemical properties of the synthesized NPs.

2.3.1. XRD

The Fe-Zn-Al-Oxide NPs formed through electrochemical synthesis were examined using the two-theta (Bragg-Brentano) coupled mode of X-ray diffraction (XRD) technique to investigate the phase structure, crystallinity, and crystallite size of the nanoparticles. The

phase structure was determined based on the diffraction results and compared with standard

data, while crystallite size was calculated using the Scherrer formula.

2.3.2. FESEM-EDS

Microstructure and surface properties of synthesized Fe-Zn-Al oxide nanoparticles were investigated by means of Field Emission Scanning Electron Microscopy (FESEM). At high vacuum, the surface morphology of the sample was observed with an accelerating voltage of 20 kV, working distance of 10.5 mm, and magnification of $\times 1100$ with Secondary Electron Detector (SED). The camera was run for 30.0 seconds live time (30.63 seconds real time), dead time 2.0%, 1791 counts per second (CPS) to obtain an image with the optimal surface morphology and distribution of particles. Consequently, the FESEM image could be observed and analyzed regarding the particle morphology, particle size, agglomerates, surface roughness and other properties of the studied Fe-Zn-Al oxide NPs. Elemental composition was detected with FESEM images provided with attached Energy Dispersive X-Ray Spectroscopy (EDS) detector.

2.3.3. FTIR



Fourier Transform Infrared (FTIR) Spectroscopy was used to determine the type of absorption bands inherent in the 400-4000 cm⁻¹ range. The spectroscopy process was done to identify functional groups, metal-oxygen bonding, and any surface chemistry present. The sample, which was prepared by electrochemical means, was analyzed through Fourier Transform Infrared (FTIR) Spectroscopy using the Bruker FTIR spectrometer. The results of the FTIR Spectroscopy showed resemblance with the FTIR spectra of mixed iron, zinc, and aluminum oxides with water of hydration and some carbonate/organic contamination.

2.3.4. UV-Vis Spectroscopy

The optical properties of the NPs under UV visible light conditions were evaluated by using the UV-visible Spectrophotometer (Simadzu, UV-1800) through measurement of the absorption spectrum. The UV-Visible Spectroscopic analysis of the samples was performed at a wavelength range of 200-800nm with slit width and step size of 1.0nm each and average scan rate according to the standard auto-scan method. The analysis was done using the UVProbe software version 2. 70.

2.3.5. Fungal Colonization and Antifungal Efficacy of Electrochemically Synthesized Fe–Zn–Al Oxides

2.3.5.1. Agar Well Diffusion Method

Antifungal efficacy of the prepared Fe–Zn–Al Oxide NPs was assessed against *Penicillium*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Rhizopus* sp. by agar well diffusion technique as shown in Fig. 2. Potato Dextrose Agar (PDA) was prepared and sterilized according to the manufacturer's recommendations and aseptically poured in sterile Petri dishes to make solidified medium. The media were also incubated without inoculating to confirm their sterility. Standardized inocula of each test organism were streaked evenly on PDA medium using 200 µL of inoculum and sterile glass spreader. Wells of about 6 mm were bored using sterile cork borer, and 200, 400, 600, 800, 1,000, 1,200, 1,400, and 1,600 µg/mL concentrations of Fe–Zn–Al nanoparticle suspensions were introduced. Distilled water was used as negative control. The plates were incubated at 28 °C, and antifungal efficacy was determined in terms of inhibition zone diameter (mm). [18], [19]

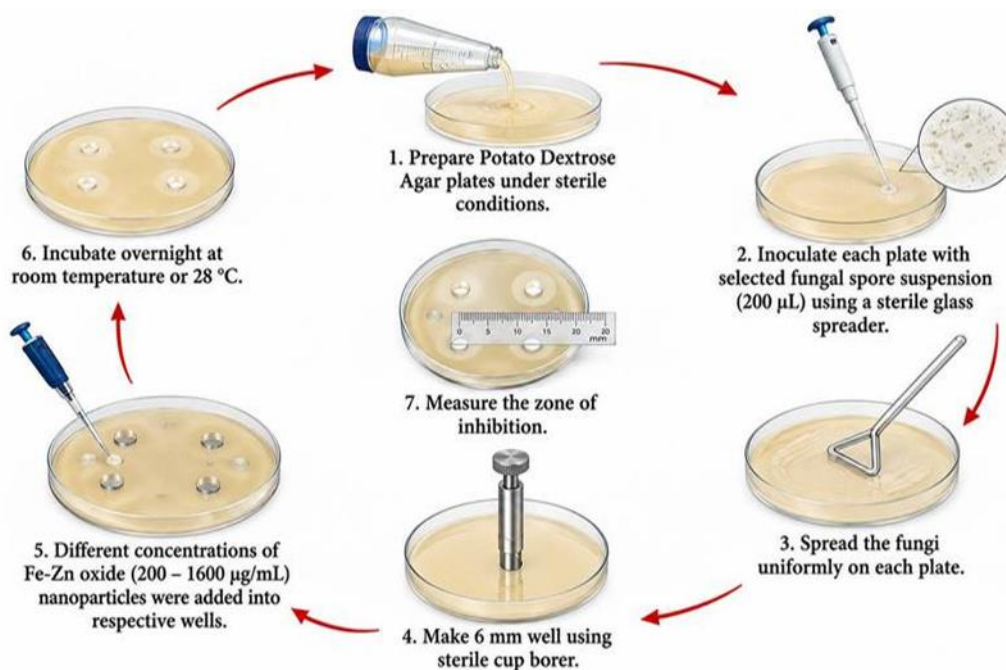


Figure 2. Flow chart of Agar well diffusion assay

2.3.5.2. Gravimetric Measurement

Dry-Biomass

The antifungal efficacy of the synthesized iron-zinc-aluminum oxide nanoparticles is quantitatively presented in Figure 3, which shows the determination of dry biomass weight of *Penicillium*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Rhizopus* sp. produced in liquid culture medium of PDB. The gravimetric technique directly determines the growth of mycelia of fungi by collecting and weighing the dried biomass after exposure to various concentrations of the test material. In sterilized test tubes containing 8 ml PDB, Fe-Zn-Al oxide nanoparticles were added at concentrations of 200, 400, 600, 800, 1,000, 1,200, 1,400, and 1,600 µg/ml. Negative controls contained untreated cultures with PDB and fungal spores. Spore suspension of the fungus

was inoculated in each test tube at a concentration of 5×10^5 spores/ml and incubated at 28 °C for 48 hours in a shaking condition. After incubation, fungal mycelial biomass was collected by vacuum filtration using weighed Whatman No. 1 filter paper and dried at 60°C to constant mass. The dried biomass and filter paper were weighed, and net fungal biomass was calculated by subtracting the initial filter-paper mass. Percentage biomass reduction was calculated as $\% \text{ biomass reduction} = \frac{(W_c - W_t)}{W_c} \times 100$, where W_c is the dry biomass of the untreated control and W_t is the biomass of the treated culture. All treatments were performed in triplicate, and results were expressed as mean $\pm$ standard deviation. [20-24]



COMMANDER Sample ID (Coupled TwoTheta/Theta)

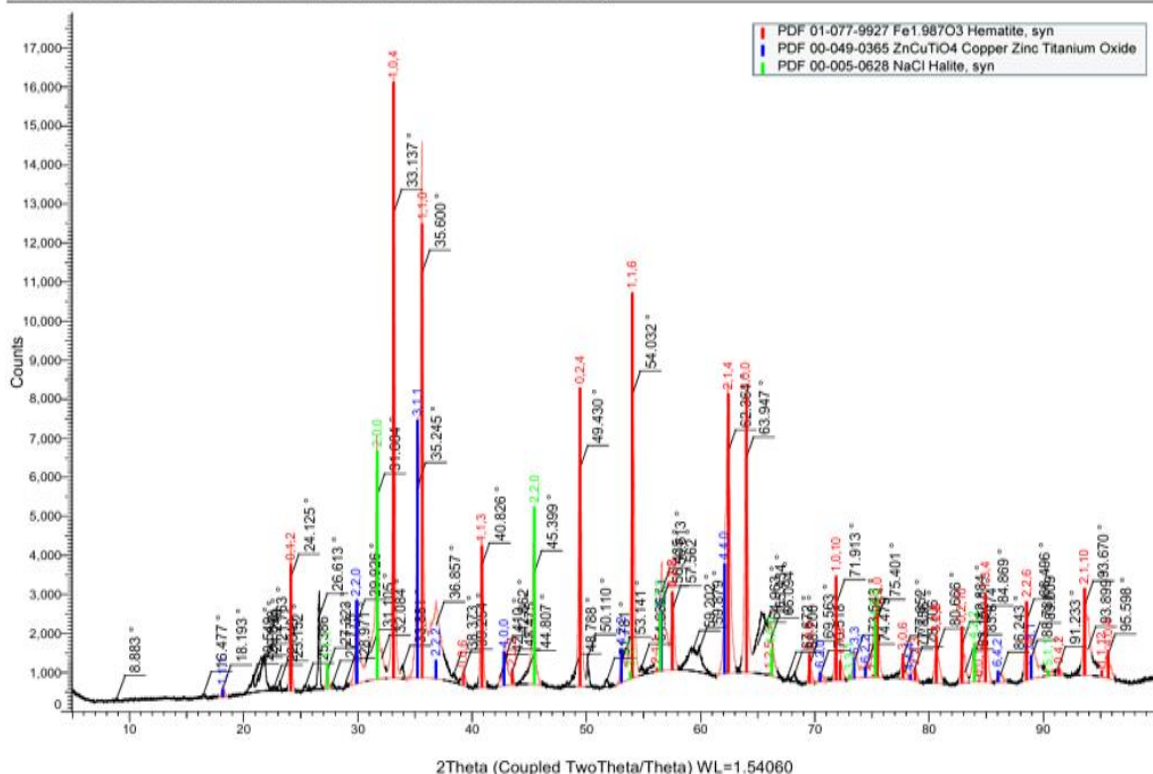


Figure 3. Flow chart of Gravimetric Dry-Biomass Measurement

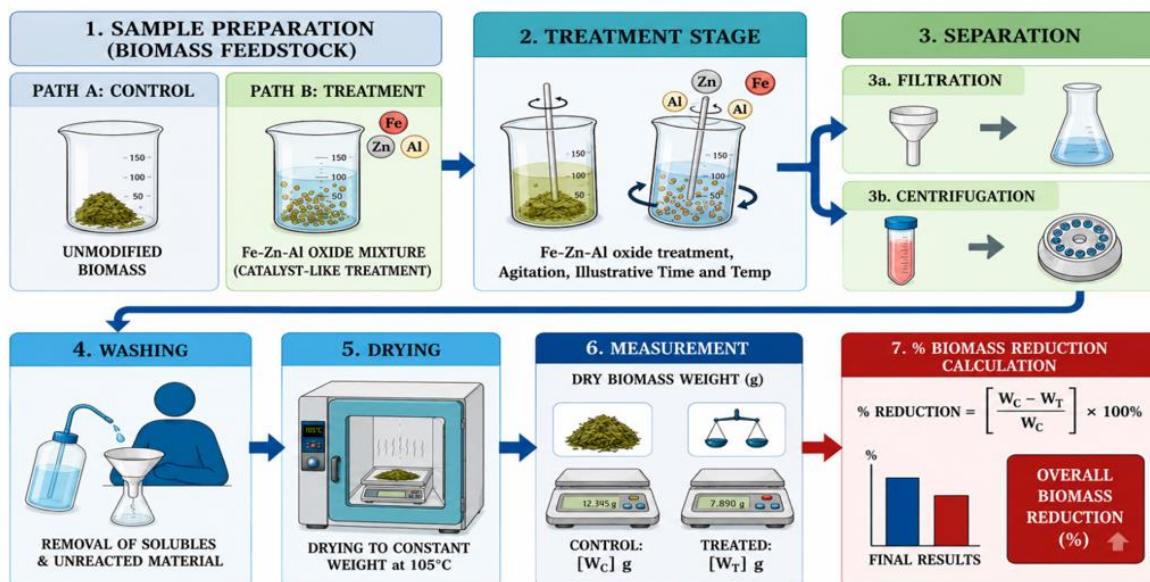


Figure 4. X-ray diffraction of Fe-Zn-Al Oxide3.Result and Discussion



3.1. Structural Characterization

3.1.1. X-ray Diffraction (XRD)

The XRD data presented in Figure 4, were used for the identification of crystalline phases of Fe-Zn-Al mixed oxide NPs prepared in this study. The sharp and broad XRD peaks at 2θ angles of 33.14, 35.60, 49.43, 54.03, 62.47, 63.95, 71.91, 74.78, 91.21, and 95.59° corresponded to the following α -Fe₂O₃ crystalline planes; (012), (104), (110), (113), (024), (116), (214), (300), (1010) and (220). Thus, the major crystalline phase is hematite. The weak ones at 2θ angles of 31-32°, 36.3°, 56-57°, 62-63° and 66-68° correspond to the formation of Zn containing spinel phases upon the introduction of Zn²⁺ and Al³⁺ ions into the tetrahedral and octahedral sites of α -Fe₂O₃ respectively. This result indicated a highly crystallized sample with nanocrystalline structure. The absence of ZnO and α -Al₂O₃ characteristic peaks suggested the incorporation of both Zn and Al ions into the hematite matrix to form mixed oxides with perovskite structure that would improve the catalytic and antibacterial properties of the product. There was evidence of peak formation on the diffractogram in accordance with the characteristics of the crystalline form of α -Fe₂O₃ for angles 2θ equal to 24.13, 33.14, 35.60, 40.83, 49.43, 54.03, 62.47, 63.95, 71.91, 74.78° and the Miller indices (012), (104), (110), (113), (024), (116), (214), (300), (1010), (220) for the crystalline plane. High angle

peaks appeared for 2θ equal to 91.21 and 95.59° and confirmed the crystallinity of hematite. Moreover, some low-intensity peaks have been detected as the peaks of spinel metal oxide with zinc. By using the match search approach, the closest match to the structure of metal ions was predicted to be CuZnTiO₄.

3.1.1.1. Crystallite size

The crystallite sizes estimated from the prominent XRD reflections, shown in **Table 2**, (**Table 2.1 in the supplementary material**) ranged from 18.47 to 50.92 nm, with an average value of approximately 32.32 nm. These values were calculated using the Scherrer equation, $= \frac{K\lambda}{\beta \cos \theta}$, where: D = crystallite size, K = 0.9, λ = 1.5406 Å, β = FWHM in radians and $2\theta/2$. The FWHM (β) was used as a measure of diffraction-line broadening. The differences seen in the size of the crystallites for different reflections suggest that the crystal structure is anisotropic and can also result from lattice defects and microstrain. Broad peaks usually imply smaller domains of coherent scattering, while narrow peaks suggest large domains of coherent scattering. The Scherrer method gives an approximation of the coherent scattering domain size and not the particle size. [25], [26] The obtained nanoscale crystallite dimensions confirm the nanocrystalline character of the synthesized oxide and are consistent with the broadening observed in the XRD pattern.

Table 2. XRD peak positions, FWHM, and Scherrer crystallite sizes of the synthesized oxide.

No.	Miller plane	θ (°)	FWHM, β (°)	β (rad)	Crystallite size, D (nm)
1.	(104)	16.569	0.1971	0.003440	42.05
2.	(110)	17.800	0.2364	0.004126	35.29
3.	(113)	20.413	0.2193	0.003828	38.65
4.	(024)	24.715	0.2662	0.004646	32.85
5.	(116)	27.016	0.2850	0.004974	31.29
6.	(018)	28.781	0.1780	0.003107	50.92
7.	(214)	31.182	0.4659	0.008131	19.93
8.	(300)	31.974	0.3158	0.005512	29.65
9.	(10 10)	35.957	0.3976	0.006939	24.68
10.	(220)	37.240	0.5402	0.009428	18.47

3.2. Morphological Characterization and Elemental composition

3.2.1. Field Emission Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy (FESEM-EDS)

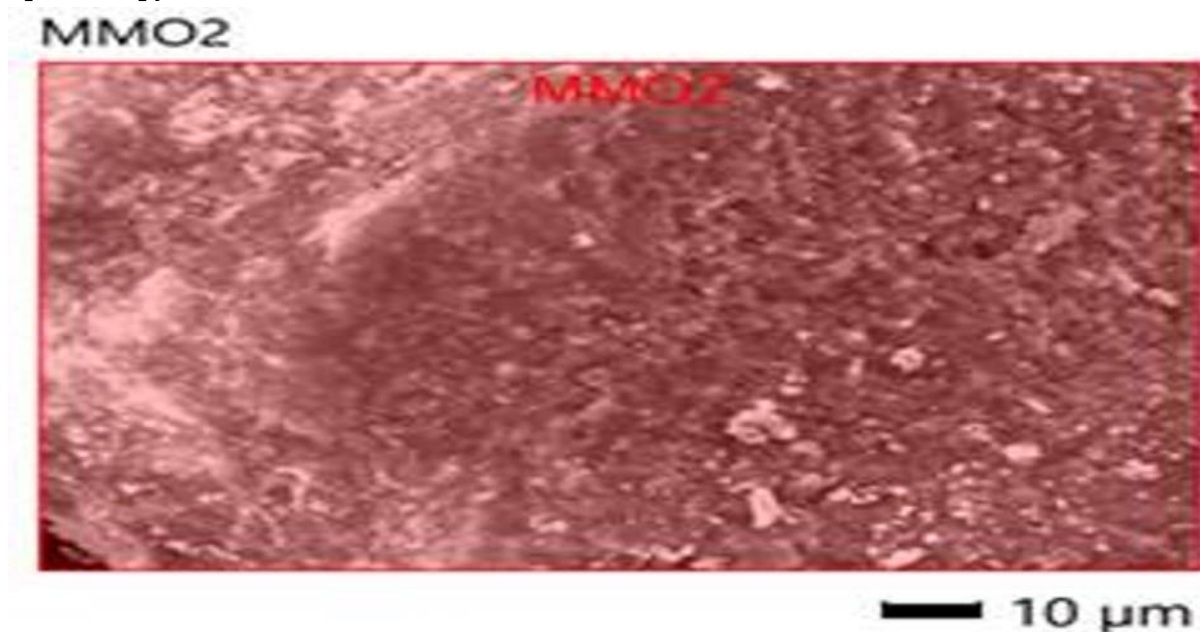


Figure 5. FESEM micrograph of synthesized Fe-Zn-Al oxide NPs

3.2.2. FE SEM–EDS Elemental Mapping Analysis of Fe–Zn–Al Oxide

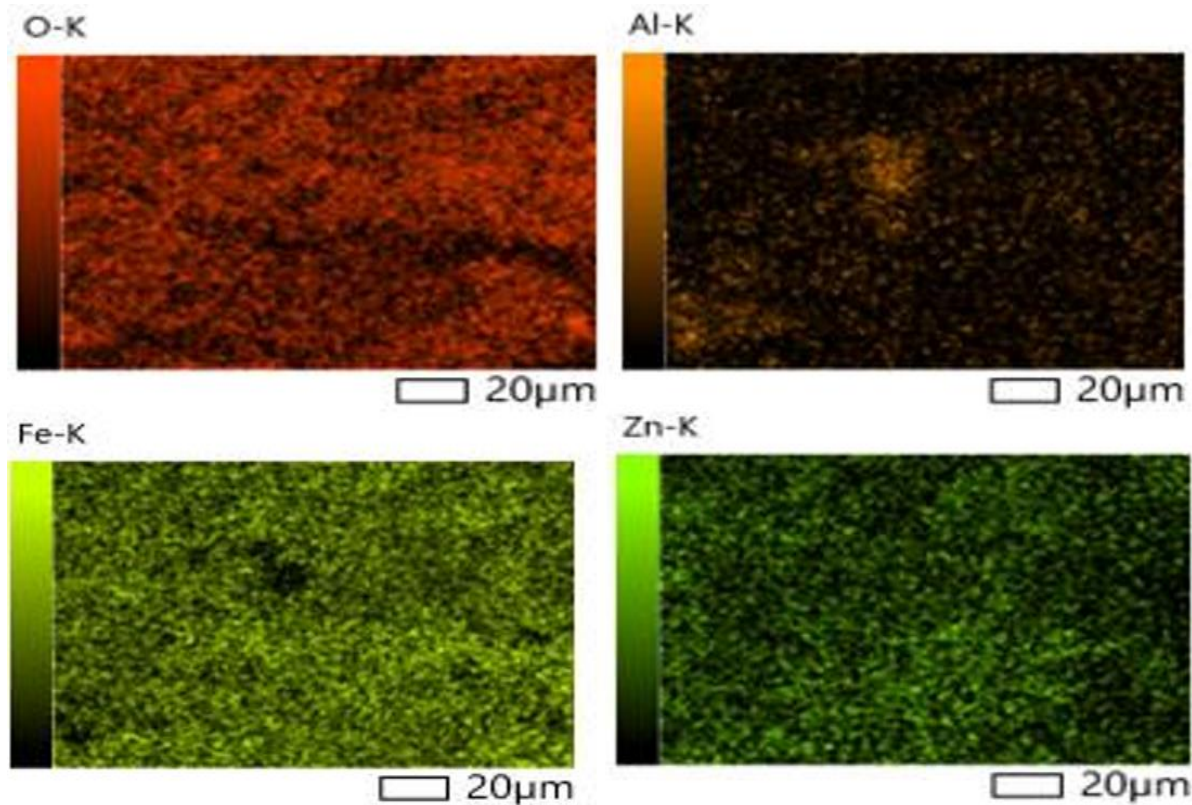


Figure 6. FESEM–EDS elemental mapping of synthesized Fe–Zn–Al oxide NPs

The elementary map presented in **Figure 5** was used to prove the synthesis of Fe–Zn–Al oxide NPs and their distribution in the surface layer of the studied sample. The elemental map results revealed the presence of oxygen (O), aluminum (Al), iron (Fe) and zinc (Zn) in the synthesized NPs. First, oxygen (O–K) was detected by EDS with a uniform distribution on the surface of the sample studied. It indicates the presence of metal oxides in the NPs studied. According to EDS analysis, the signal intensity of iron (Fe–K) was found to be the highest, and iron was evenly distributed on the surface of the examined area without any bright spots indicating the presence of metallic iron. Elemental mapping of zinc (Zn–K) revealed a less intense signal than that of Fe–K and zinc was uniformly distributed over the surface of the examined area without any bright spots. As the elemental mapping results indicate as shown in **Figure 6**, Zn atoms were included in

the structure of metal oxides. According to the EDS analysis of aluminum (Al–K), the signal intensity was less intense than that of Zn–K and Fe–K distributed on the surface of the examined area without any bright spots. This means that the NPs formed were composed of metal oxides with a low percentage of Al. FESEM–EDS elemental mapping showed that the produced NPs contained Fe, Zn, Al and O elements which were uniformly distributed on the surface of the studied sample. The produced NPs were found to contain Fe, Zn and Al oxides. The Absence of bright spots indicates the formation of NPs containing a uniform distribution of metals. Uniform distribution of metals in the formed NPs could improve the catalytic and antimicrobial activity of NPs due to an increased number of active centers.

3.2.3. EDS Spectra and Elemental composition

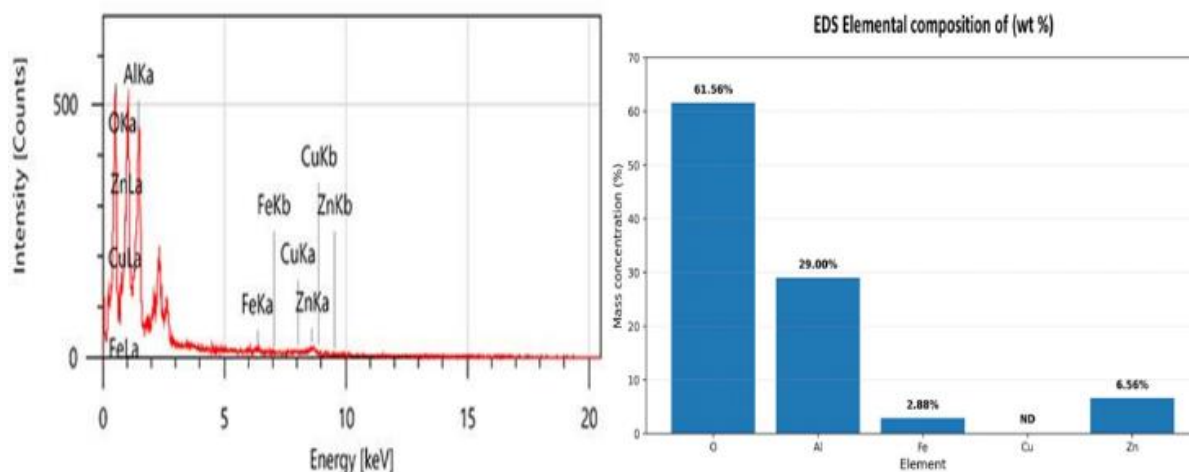


Figure 7. EDS Spectra and Elemental composition

The findings shown in **Figure 7**, from the EDS indicate that there is an indication of formation of Fe-Zn-Al oxide mixture. However, at the same time, we see that the main components present on the surface are oxygen and aluminum. The presence of oxygen is caused by the formation of metal oxides, while the presence of Fe and Zn shows that these metals have entered the oxide network. However, it should be noted that EDS is used to detect the composition of a small area covered by a thin film. EDS also has low sensitivity to light elements and non-uniform distribution. poor sensitivity for light elements and non-uniform distribution. To know the elemental composition of the synthesized Fe-Zn-Al oxide NPs, FESEM combined with EDS elemental mapping was used. It could be observed from the oxygen map that the distribution of this element was quite even. This proved the fact that the oxidation process had occurred in the synthesis and hence, the formation of the mixed

oxides. Of all the metals, it could be observed that the elemental composition of iron was the highest, and also, the distribution was even. This means that iron was the metal that underwent the oxidation process to form the oxides. The Elemental composition of aluminum was low due to its initial presence in the mixture; however, it was also rather uniform, with the presence of several areas of higher concentration. We can therefore assume that the aluminum has oxidized and entered the composite material. So, mixed oxide of Al, Zn, and Fe obtained was homogenous in terms of its composition because thermal treatment helped oxidize all the metal particles and mix them thoroughly. In this way, the explanation for the increased antifungal properties of the obtained material may lie in the high number of active sites on its surface and the sufficient formation of reactive oxygen species; this hypothesis can be correlated by XRD analysis of the mixed oxide of Fe, Zn and Al.

3.3. Optical Spectroscopic Characterization

3.3.1. Fourier Transform Infrared Spectroscopy (FTIR)

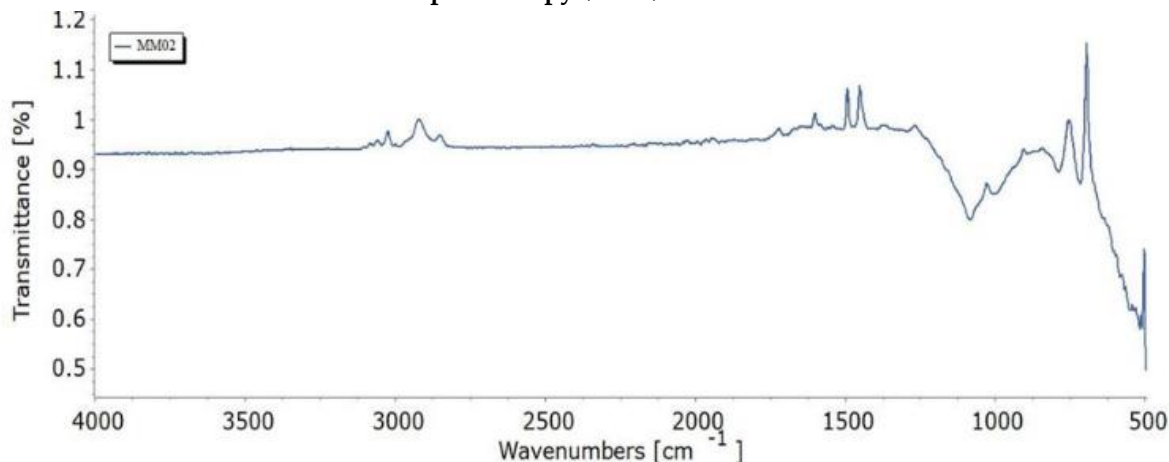


Figure 8. FTIR spectroscopy of Fe-Zn-Al oxide

Table 3. FTIR Analysis of MM02 (Fe-Zn-Al Mixed Oxide NPs)

Wavenumber (cm ⁻¹)	Band Assignment	Interpretation
~3000–2850	Weak C–H stretching	Trace organic residues from synthesis or environmental contamination.
~1630–1550	H–O–H bending vibration	Adsorbed water on the nanoparticle surface.
~1450–1380	Carbonate (CO ₃ ²⁻) or C–O vibrations	Minor atmospheric carbonate species adsorbed during storage.
1085.33	C–O stretching / M–O–H vibration	Surface hydroxyl groups and metal–oxygen–hydroxyl bonding.
589.44	Fe–O and Zn–O lattice stretching	Characteristic vibration confirming the formation of crystalline mixed metal oxide NPs.

FTIR spectroscopy analysis of the prepared Fe-Zn-Al mixed oxide NPs gave rise to peaks shown in **Figure 8** and **Table 3** that were characteristic of metal oxides. The peaks observed at 3000-2850 cm⁻¹ were due to C-H stretching while those at 1600 cm⁻¹ belonged to water bending vibrations. Another weak peak at 1400 cm⁻¹ was attributed to carbonate molecules adsorbed from the atmosphere. The peak observed at 1085.33 cm⁻¹ was assigned to C-O stretching along with bending vibrations of surface metal hydroxyl groups.

Additionally, the group observed at 589.44 cm⁻¹ was due to Fe-O and Zn-O stretching which proved the crystallization of a mixed oxide NPs of Fe-Zn-Al. The absence of organic functional groups in the FTIR spectrum of the prepared product indicated that the calcination process had effectively removed any organic residue giving rise to a mixed metal oxide. This was further proven through FESEM/EDS analysis where the EDS spectra revealed the presence of Fe, Zn, Al and oxygen in the prepared sample.

3.3.2. UV-Visible Spectroscopic analysis of Iron-Zinc-Aluminum Oxide NPs

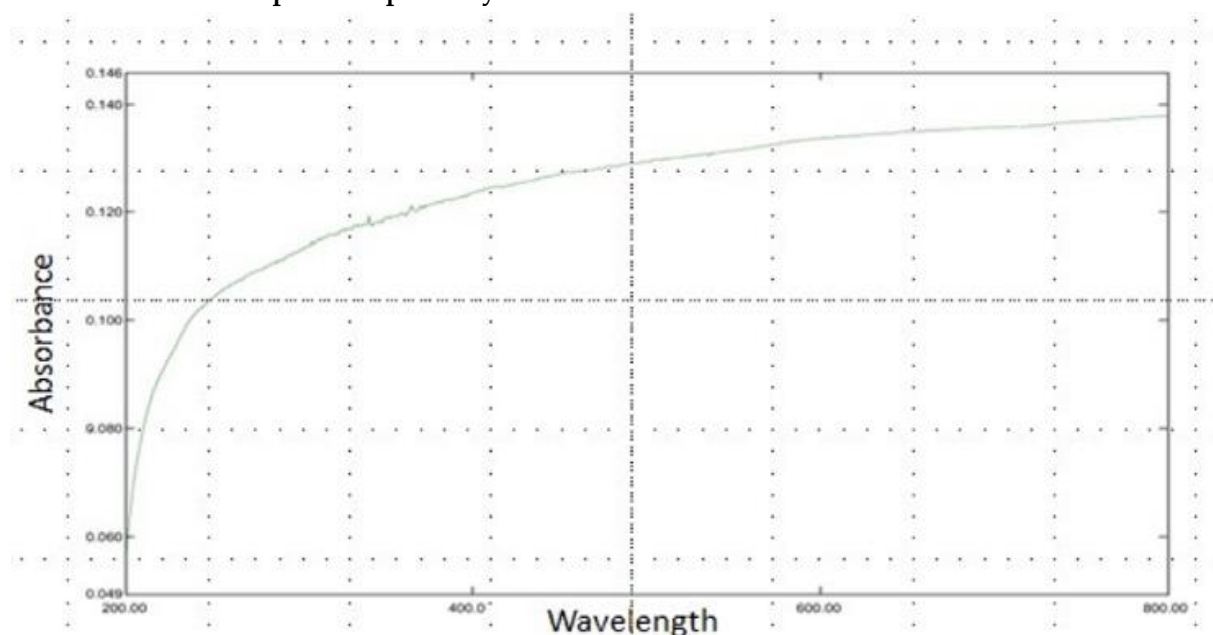


Figure 9 UV-Visible Spectroscopic graphUV-

Visible absorption spectra shown in **Figure 9** were taken using a Perkin-Elmer spectrophotometer in the wavelength range of 200–800 nm to study the optical properties of iron-zinc-aluminum oxide NPs, shown in Fig. 1. Initially, it is possible to notice a slight absorbance limit from 200 nm, corresponding to the UV region. In the wavelength range 200–800 nm, the absorption curve increases slowly, without visible absorption peaks. The absorption value goes from 0.06 at a wavelength of 200 nm to 0.14 at a wavelength of 800 nm, which means that this material has broadband absorption properties.

The absence of obvious excitonic absorption peaks means that the studied sample is mainly polycrystalline in nature and includes a combination of NPs with a specific size distribution. Broadband absorption is characteristic of materials that include different types of atoms, such as Fe, Zn, Al, and O atoms, each contributing to optical absorption through specific electronic transitions.

Since the absorption curve continues to extend to the visible part of the spectrum, this implies

that the materials will have enhanced light-harvesting ability, which is good for photocatalytic and antimicrobial activities and environmental purposes. The optical spectrum is smooth, indicating that there is a homogeneous iron-zinc-aluminum oxide and not individual metal NPs formed as shown in the XRD and microscopy tests. Therefore, the optical results are consistent with the synthesis of iron-zinc oxide NPs with defective surfaces and broad optical absorption. In conclusion, the UV-Vis spectrum shows that the synthesized iron-zinc-aluminum oxide NPs exhibit extensive absorption in the UV and visible region, which is typical of nanoscale metal oxides and could be used in photocatalytic and antimicrobial activities.[27]

3.3.3. Optical Band Gap Analysis

From the linear fitting equation in the Tauc plot, $(\alpha h\nu)^2 = 0.2946 (h\nu) - 0.1794$. The optical band gap is obtained by extrapolating the linear portion to $(\alpha h\nu)^2 = 0$: This is also proved by extrapolating the Tauc plot.

$$E_g = \frac{0.1794}{0.2946} \approx 0.61 \text{ eV}$$

The Tauc plot exhibits a reasonably linear absorption region, with $R^2=0.906$, indicating a good fit to the direct allowed transition model. The estimated optical band gap of ~ 0.61 eV suggests relatively low-energy electronic transitions in the synthesized material. The

deviation from linearity near the absorption edge may arise from defect states, structural disorder, particle-size effects, or overlapping electronic transitions. See **Figure 10**. Overall, the plot proves semiconducting optical behavior with a direct optical transition.

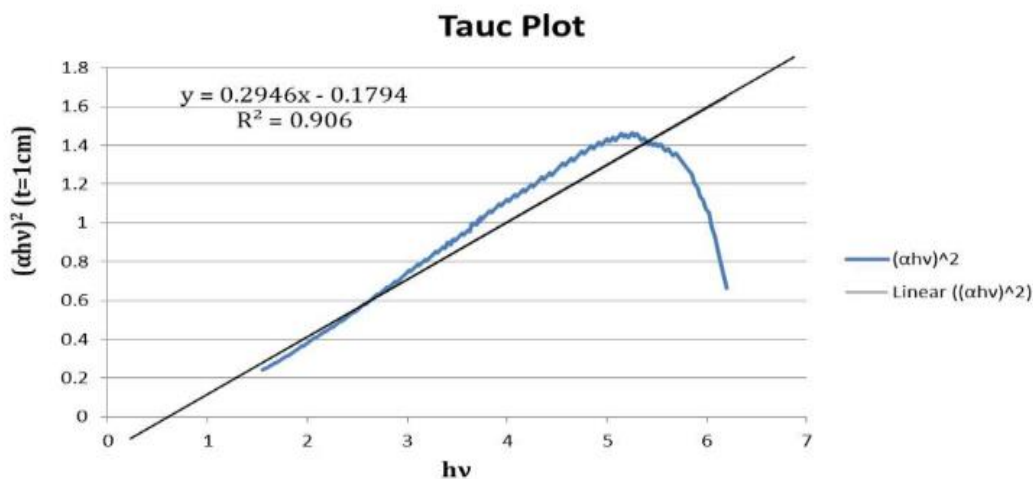


Figure 10 Tauc Plot band gap of Fe-Zn-Al Oxide NPs3.4.

Antifungal Evaluation of

electrochemically synthesized Fe-Zn-Al Oxide against fungal colonies

3.4.1. Antifungal Evaluation of electrochemically synthesized Fe-Zn-Al Oxide against fungal colonies using Agar Well Diffusion Assay

The diffusion test in Agar wells was carried out to evaluate the antifungal activity of the synthesized iron-zinc-aluminum oxide NPs against *Rhizopus* sp, *Penicillium*, *Aspergillus niger* and *Aspergillus fumigatus*. It is possible to consider that the reasons for the differences in antifungal activity can be attributed to the structural characteristics of the cell walls and

membranes of the fungi and their sensitivity to reactive oxygen species generated during interaction with the mixed metal oxide pigment. The increasing dependence of inhibition zones on increasing concentration of the tested pigment is possibly related to increased contact between pigment particles and fungi. In this case, the lack of activity towards *Rhizopus* sp. and *Aspergillus fumigatus* **Figures 11 and 12** can be justified by the low diffusibility of pigment particles in agar and the weak antimicrobial properties towards this fungus under oxidative stress conditions.

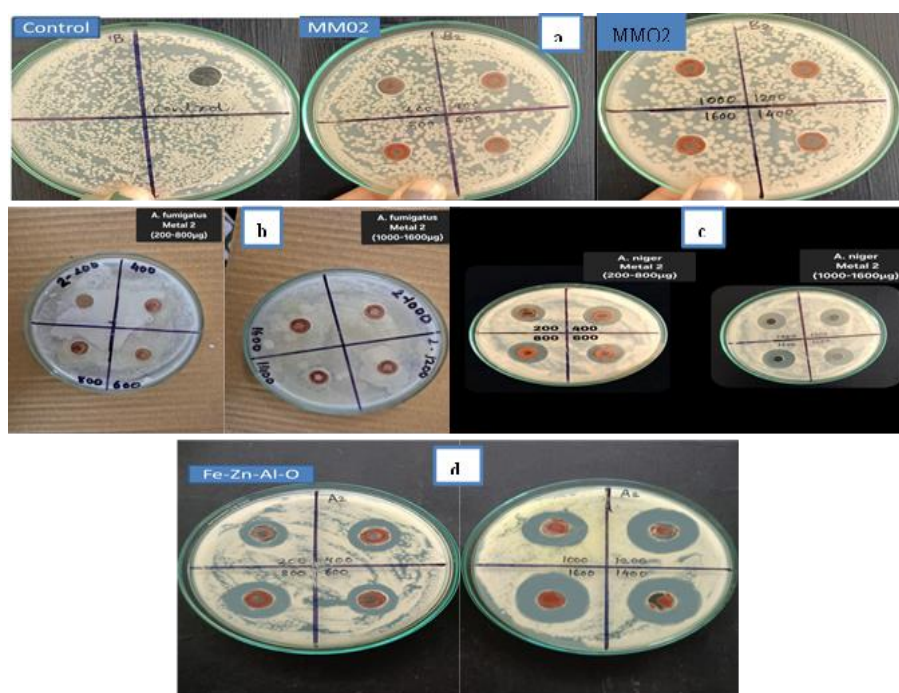


Figure 11. Inhibition effect of Fe-Zn-Al oxide on (a) *Rhizopus* Sp., (b) *A. Fumigatus*, (c) *A. Niger* and (d) *Penicillium*

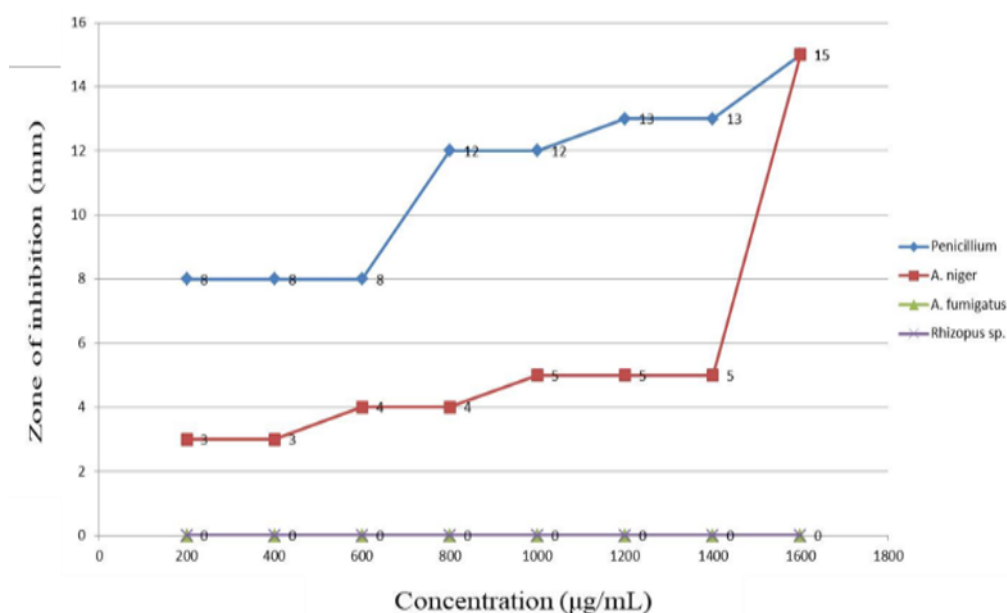


Figure 12. Zone of inhibition against different fungal species at varying pigment concentrations using Agar Well Diffusion Assay

Among all fungal strains tested, *Penicillium*, as shown in Figures 11(d) and 12 (Table 5 in Supplementary Material), was found to be the most affected by Fe-Zn-Al oxide NPs. The

inhibition zone of 8 mm was detected at the concentration of 200µg/mL, and its increase to 600µg/mL did not affect the result. Increasing the concentration to 800–1000µg/mL resulted in an increase in the inhibition zone to 12 mm, to

13 mm at the concentration of 1200–1400 µg/mL, and to 15 mm at the concentration of 1600 µg/mL. The zone of inhibition of the fungus *Aspergillus niger* shown in **Figure 11(c)**, was 3 mm at the two lowest nanoparticle concentrations. However, using concentrations of 600 to 800 µg/mL increased to 4 mm, 5 mm for 1000 to 1400 µg/mL, and 6 mm for 1600 µg/mL. Thus, it is possible to conclude that the sensitivity of this fungus to the effect of NPs is lower than that of *Penicillium* but higher than that of *Rhizopus* sp. On the contrary, there are *Rhizopus* sp. **Figure 11(a)** and *Aspergillus fumigatus* **Figure 11(b)** fungi which were not affected by any of the concentrations tested (200–1600 µg/mL). There is no appearance of inhibition zone for nutrient agars with Fe–Zn–Al oxide NPs at any of the concentrations used, which proves the lack of effectiveness of the synthesized NPs against *Rhizopus* sp.

Penicillium > *Aspergillus niger* >> *Rhizopus* Sp.
= *Aspergillus fumigatus*

In this way, the result proves that iron-zinc-aluminum oxide NPs had some antifungal activity against *Penicillium* and *Aspergillus niger*, while they were ineffective against *Rhizopus* sp. and *Aspergillus fumigatus*. Furthermore, the obtained results clearly show that the higher the concentration of the NPs, the higher the antifungal activity of the tested NPs will be, probably due to the production of reactive oxygen species and disruption of the integrity of the cell membrane of fungi.

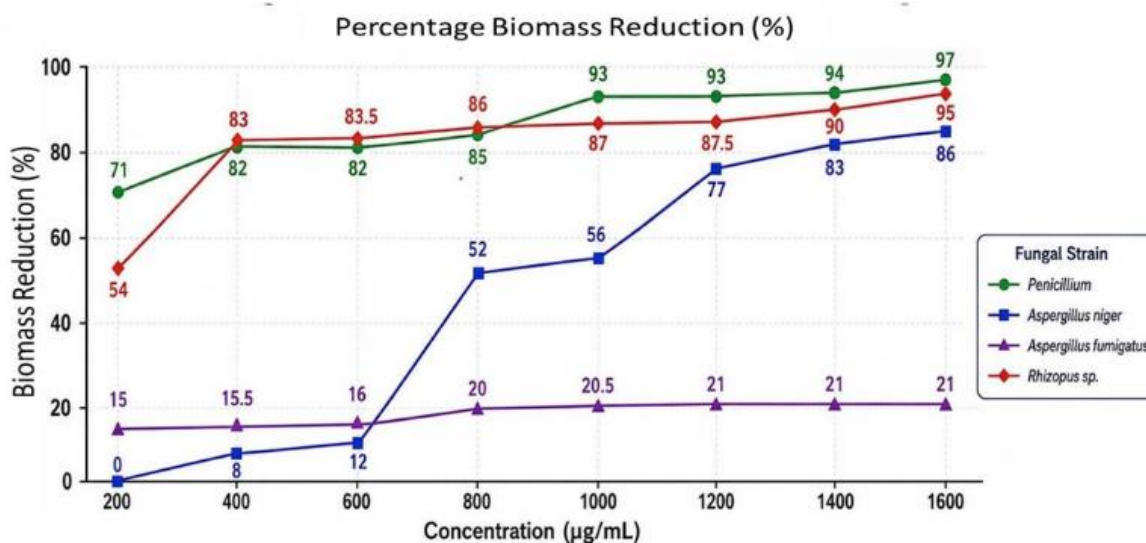
3.4.2. Antifungal Evaluation of electrochemically synthesized Fe–Zn–Al Oxide against fungal colonies using gravimetric dry-biomass measurement method

The antifungal effect of Fe–Zn–Al oxide NPs was evaluated using gravimetric measurement of dry biomass at concentrations ranging from 200 to 1600 µg/mL. As shown in **Figure 13**

(**Table 6** in Supplementary Material), the percent reduction in fungal biomass demonstrated a concentration-dependent reduction effect against all four fungal pathogens tested, although the magnitude of biomass reduction varied considerably among species. *Penicillium* spp. exhibited the greatest susceptibility, with biomass reduction increasing from 71% at 200 µg/mL to 97% at 1,600 µg/mL. *Rhizopus* sp. also showed pronounced sensitivity, with a biomass reduction of 54% at the lowest concentration and 95% inhibition at 1600 µg/mL. Notably, concentrations of 400 µg/mL and above resulted in a >80% reduction in *Rhizopus* biomass, indicating substantial suppression of mycelial development. These findings demonstrate the strong concentration-dependent antifungal potential of the Fe–Zn–Al oxide NPs, particularly against *Penicillium* spp. and *Rhizopus* sp.

In contrast, *Aspergillus niger* exhibited comparatively lower sensitivity at the lower concentrations. No measurable reduction in biomass was observed at 200 µg/mL, while the reduction in biomass increased slightly to 8–12% at 400–600 µg/mL. A marked increase was observed at 800 µg/mL, where biomass reduction reached 52%, subsequently increasing to 86% at 1,600 µg/mL. *Aspergillus fumigatus* was the least susceptible organism, showing approximately 15% inhibition at 200 µg/mL and reaching only 21% biomass reduction at 1,200–1,600 µg/mL. Based on maximum biomass reduction, the susceptibility pattern was:

Penicillium spp. (97%) > *Rhizopus* sp. (95%) > *A. niger* (86%) >> *A. fumigatus* (21%). (21%). Overall, gravimetric results demonstrate strong antifungal activity of Fe–Zn–Al oxide NPs against *Penicillium* spp. and *Rhizopus* sp. ,



substantial activity against *A. niger*, and relatively limited activity against *A. fumigatus*,

highlighting the differences in sensitivity depending on the species.

Figure 13. Antifungal Activity of Fe-Zn-Al Oxide NPs gravimetric dry-biomass measurement

3.4.3. Comparative Evaluation of the Antifungal Activity of Fe-Zn-Al Oxide NPs by Agar Well Diffusion and gravimetric dry-biomass measurement Methods

The agar well diffusion and gravimetric dry-biomass assays produced substantially different antifungal response patterns for the Fe-Zn-Al oxide NPs, highlighting the importance of the biological endpoint and assay principle. In the agar well diffusion assay, antifungal activity was primarily evident against *Penicillium* spp. and *Aspergillus niger*, with maximum inhibition zones of 15 and 6 mm, respectively, while no measurable inhibition zones were detected for *Rhizopus* sp. or *Aspergillus fumigatus*. The corresponding susceptibility profile was therefore *Penicillium* spp. pattern was therefore

***Penicillium* spp. > *Aspergillus niger* >> *Rhizopus* sp. = *Aspergillus fumigatus*.**

In contrast, gravimetric analysis demonstrated substantial inhibition of mycelial biomass in all four fungi, with maximum reductions of 97% for *Penicillium* spp. 95% for *Rhizopus* sp. 86 % for *Aspergillus niger* and 21 % for *Aspergillus*

fumigatus. Thus, the gravimetric test revealed considerable antifungal activity against *Rhizopus* sp. was not apparent in the agar diffusion assay.

The contrasting response of *Rhizopus* sp. is particularly important when interpreting the antifungal performance of the Fe-Zn-Al oxide NPs. The absence of a zone of inhibition does not necessarily indicate a lack of antifungal activity because diffusion into the agar wells is highly dependent on the ability of the test material to migrate through the agar matrix. Mixed metal oxide NPs may exhibit limited diffusion due to their particle size, aggregation, surface characteristics, and interactions with the agar matrix. [28], [29] Consequently, NPs can exert substantial antifungal effects through direct contact with fungal cells while producing little or no measurable diffusion zone. Gravimetric analysis, on the other hand, assesses the cumulative effect of nanoparticle exposure on fungal biomass and therefore depends less on particle diffusion. The 54% biomass reduction observed for *Rhizopus* sp. at 200µg/mL and the increase to 95% at



1,600µg/mL provide strong evidence of concentration-dependent suppression of mycelial development.

Overall, the two assays should be regarded as complementary rather than contradictory. Both methods identified *Penicillium* spp. as the most susceptible fungus, with a 15-mm inhibition zone and 97% biomass reduction at 1,600µg/mL. *A. niger* also showed consistent sensitivity, although the magnitude differed between trials. *A. fumigatus* remained the least responsive, showing no diffusion zone but approximately 21% biomass inhibition at the highest concentrations. The significantly stronger gravimetric response of *Rhizopus* sp.

demonstrates that diffusion on agar alone can underestimate the antifungal activity of poorly diffusible mixed metal oxide NPs. The combined results suggest that Fe–Zn–Al oxide NPs possess concentration-dependent antifungal activity, with species-specific differences as shown in **Table 4**, potentially related to fungal cell wall and membrane characteristics, particle cell interactions, and mechanisms of oxidative stress, including the generation of reactive oxygen species.[30], [31] Therefore, gravimetric measurement of biomass provides valuable quantitative confirmation of antifungal activity that may not be captured by diffusion in agar wells alone.

Table 4. Comparative Summary of % inhibition using Agar Well Diffusion and %gravimetric biomass reduction methods

Fungal species	Agar Well Diffusion (mm)	% gravimetric biomass reduction	Comparative interpretation
<i>Penicillium</i>	Maximum inhibition zone ≈ 15 mm	97.%	Highly susceptible in both methods; biomass reduction reveals much stronger activity.
<i>Rhizopus</i> sp.	Very small or no inhibition zones	95.%	Strong biomass reduction due to enhanced nanoparticle contact.
<i>Aspergillus niger</i>	Moderate inhibition only at higher concentration	86.%	Moderate susceptibility; higher concentrations required for effective inhibition.
<i>Aspergillus fumigatus</i>	Very small or no inhibition zones	21.%	Least susceptible species in both assays, indicating inherent resistance.

4. Conclusion

From this experiment, it was shown that there is the potential for producing multifunctional mixed metal oxides pigment from scrap metals having iron, zinc, and aluminum using EC synthesis process followed by heat treatment and mechanical blending. It provides a very easy way for utilization of scrap metals without requiring the use of precursor compounds. The structural and physicochemical

characterization demonstrated the formation of highly crystalline and nanostructured oxide material. XRD characterization indicated the presence of hematite as the major crystalline phase along with minor zinc oxide spinel phases. Crystal sizes ranged between 18.47 nm to 50.92 nm showing that the resulting material is nanocrystalline.

According to FESEM results, there were irregular to quasi-spherical and elongated



nanosized structures showing significant clustering, which were expected due to high surface energy for oxide nanoparticles. EDS proved the presence of Fe, Zn, Al, and O elements in the sample along with rather homogeneous composition, while XRF showed that Fe, Zn, and Al were major components of the bulk sample. The difference in compositions from EDS and XRF further emphasizes the heterogeneous nature of the material and different sampling depths from the two types of analysis. The UV-Vis spectroscopy results showed wide absorption in the spectral range studied, while the Tauc plot gave an estimated optical bandgap of 0.61 eV, indicating semiconducting optical properties.

The antifungal results proved species-specific and methodological differences in the responses. In agar well diffusion assay, the inhibitory zones against *Penicillium* spp. and *A. niger* were 15 and 6 mm, respectively, but the inhibitory zones against *Rhizopus* sp. and *A. fumigatus* were not measurable. In contrast, the inhibitions of the fungi measured by dry biomass were much higher, 97%, 95%, 86%, and 21% against *Penicillium* spp., *Rhizopus* sp., *A. niger*, and *A. fumigatus*, respectively. In summary, the combined physicochemical and biological results suggested the feasibility of the use of recycled Fe–Zn–Al Oxides as long-lasting multifunctional pigments for water-based antifungal paints. However, further studies should optimize their compositions, dispersions, durability of the paints, antifungal efficacies, and mechanisms related to the fungicidal properties of the oxide surfaces.

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6. Disclosure Statement

The authors declare that there has been no competing interest among the authors which could influence the writing and publication of this manuscript.

7. Use of Generative AI

During the preparation of this study the author(s) used ChatGPT (Version 5.2) to improve the readability and language of the manuscript. After using this tool, the author(s) reviewed and edited the contents as needed.

8. Conflict of Interest

The authors declare that there has been no conflict of interest during the study that could influence the preparation of this manuscript.

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10. Data Availability

Data will be available on request.



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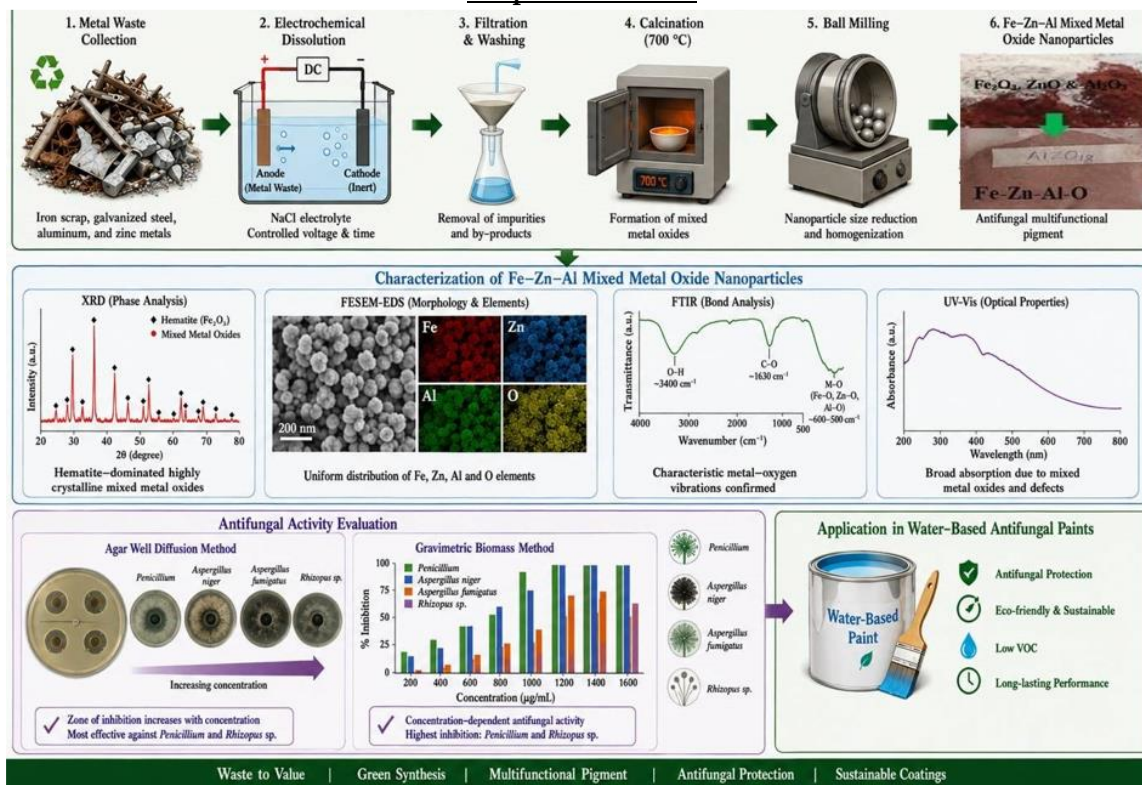


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Graphical Abstract



Supplementary Materials

Table 2.1 XRD peak positions, FWHM (β), d -spacing, and Scherrer crystallite sizes of the synthesized oxide.

No.	2 θ (°)	Miller plane	θ (°)	FWHM, β (°)	β (rad)	d -spacing (Å)	Crystallite size, D (nm)
1	33.137	(104)	16.569	0.1971	0.003440	2.7013	42.05
2	35.600	(110)	17.800	0.2364	0.004126	2.5198	35.29
3	40.826	(113)	20.413	0.2193	0.003828	2.2085	38.65
4	49.430	(024)	24.715	0.2662	0.004646	1.8424	32.85
5	54.032	(116)	27.016	0.2850	0.004974	1.6958	31.29
6	57.562	(018)	28.781	0.1780	0.003107	1.5999	50.92
7	62.364	(214)	31.182	0.4659	0.008131	1.4878	19.93
8	63.947	(300)	31.974	0.3158	0.005512	1.4547	29.65
9	71.913	(10 10)	35.957	0.3976	0.006939	1.3119	24.68
10	74.479	(220)	37.240	0.5402	0.009428	1.2729	18.47



Table 5. Result of Agar Well Diffusion Assay against selected fungi

Method	Concentration ($\mu\text{g/mL}$)	Zone of Inhibition (mm)			
		<i>Penicillium</i>	<i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i>	<i>Rhizopus sp.</i>
Agar Well Diffusion Assay	D/W	0	0	0	0
	200	8	3	0	0
	400	8	3	0	0
	600	8	4	0	0
	800	12	4	0	0
	1000	12	5	0	0
	1200	13	5	0	0
	1400	13	5	0	0
	1600	15	15	0	0

Table 6. Result of Broth dilution method (% inhibition) against selected fungi

Method	Concentration ($\mu\text{g/mL}$)	Zone of Inhibition (%)			
		<i>Penicillium</i>	<i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i>	<i>Rhizopus sp.</i>
Broth dilution method (% inhibition)	200	71	0	15	54
	400	82	8	15.5	83
	600	82	12	16	83.5
	800	85	52	20	86
	1000	93	56	20.5	87
	1200	93	77	21	87.5
	1400	94	83	21	90
	1600	97	86	21	95