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Green Synthesis of Calcium Oxide Nanoparticles by Endophytic Fungi for Sustainable Textile and Leather Wastewater Remediation

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Abstract

This study investigated the potential of endophytic fungi and calcium oxide nanoparticles **(**CaONPs**)** for bioremediation and industrial wastewater treatment. Five endophytic strains were isolated and evaluated for tolerance to heavy metals (Cu, Pb, Cd, Hg, and As) at concentrations of 25 – 100 ppm. Results indicated strain-specific tolerance patterns, with *Curvularia lunata* showing high Hg and Cd tolerance, *Fusarium solani* exhibiting exceptional Cd resistance, and *Rhizopus oryzae* tolerating moderate Hg and Pb levels, highlighting their potential for bioremediation of metal-contaminated environments. Morphological and microscopic characterization confirmed the identity of the isolates as *Curvularia lunata*, *Aspergillus flavus*, *Alternaria alternata*, *Fusarium solani*, and *Rhizopus oryzae*, supporting their selection for environmental applications. The CaONPs were synthesized and characterized, revealing high surface area and reactive morphology suitable for pollutant adsorption. The nanoparticles were applied to textile and leather wastewater**,** and treatment efficiency was evaluated under varying pH, temperature, concentration, and contact time. Optimal conditions for maximum removal were observed at neutral pH (7), moderate temperature (30 °C), intermediate concentration/dosage, and 60 minutes' contact time**.** Treated wastewater showed significant improvement ($p < 0.05$) over controls, with textile effluents generally exhibiting slightly higher response values than leather wastewater. CaONPs demonstrated effective removal of contaminants through adsorption and coagulation mechanisms, with performance influenced by physicochemical conditions. Thus, this study highlighted the complementary potential of heavy metal-tolerant endophytic fungi and CaONPs for sustainable remediation strategies. The findings provide a foundation for developing integrated biological and nanomaterial-based approaches to manage industrial effluents and mitigate environmental contamination.

Keywords: Bioremediation, Calcium oxide nanoparticles**,** Endophytic fungi, Heavy metals, Industrial effluent

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Introduction

Endophytes are defined as all microorganisms that colonize asymptomatically within living healthy tissues. In general, endophytes are considered as commensalistic symbionts, where they receive nutrients and shelter from the host. In return, they are thought to provide the host with chemical constituents that can be used in the growth or defense mechanisms. Fungal endophytes have attracted a great interest to microbiologists, chemists and ecologists as a treasure of biological resources, because they play diverse indispensable roles in the ecosystem for stress tolerance, eco-adaptation, and promoting growth and development. Therefore, they were considered as an unusual source of novel secondary metabolites, exhibiting a variety of biological activity, which are in use in modern agriculture, pharmaceutical and biotechnological industries (Hughes, 2017).

One of the major environmental problems faced by the world today is the contamination of soil, water and air by toxic chemicals. In addition, increasing contamination of

groundwater by toxic metal ions poses significant environmental hazard as they are non-degradable and can accumulate in living tissues to become concentrated throughout the food chain which leads to various diseases and disorders (Egurefa *et al*. 2020a; 2020b; Okolo *et al.*, 2025; Okpalaunegbu *et al.*, 2025; Obiefuna *et al*. 2025). The presence of pathogens, heavy metals and organic pollutants is the main indication of water contamination. Heavy metal and organic pollutant contamination of water are public health concerns with several health risks associated with it (Uba, 2019a; 2019b; 2019c; Uba *et al*. 2019b; 2019c).

The presence of some heavy metal ions like Ni^{2+}, Cr^{6+} and Pb^{2+} in water bodies in industrialized cities poses significant threat to life due to their toxic nature even at low concentrations as documented by several authors (Uba *et al.*, 2017; Nkamigbo *et al*. 2020a; 2020b; Njoku *et al*. 2019a; 2019b; Uba *et al*., 2020a; 2020b; Umeh *et al.*, 2020; 2021; Dokubo *et al*., 2022a; 2022b; Anidu *et al*., 2023; Obiefoka *et al*., 2023; Ubajekwe *et al*., 2025; Uba *et al*., 2025; Oghonim, 2023; Oghonim *et al.* 2023; 2026). The elimination of copper, lead, cadmium and iron ions from aqueous solution by classic methods includes its deposition with alkali hydroxide or lime, electrolytic precipitation, ion exchange and reverse osmosis. These classic methods are expensive and have a lot of disadvantages such as production of metal-bearing sludge or wastes, imperfect metal elimination and the elimination of secondary wastes (El – Dafrawy *et al*., 2015; Uba *et al*. 2016; 2018a; 2018b; 2018c; 2019d; 2019e). The challenges of physical and chemical techniques encountered during waste water treatment as mentioned above made biological technique an attractive technology. It was against this challenge that several conventional wastewater technologies were developed and are in use successfully at a large scale to reduce these hazardous compounds concentration in wastewaters (Alisa *et al*., 2020; Anukam *et al*., 2020a; 2020b; Uba e*t al.*, 2021a; 2021b). One of the identified reducing agents for heavy metals is the use of microorganisms like fungi. Fungi can tolerate and detoxify metals in many ways. It could be through valence transformation, active uptake, precipitation inside or outside their cells and biosorption. Biosorption is a process of metal uptake by living or dead biomass through the binding of metal ion on the cell wall and extracellular materials (Manguilimotan and Bitacura, 2018). Hence, fungi have been recognized as the best candidate for both biosorption and metal nanocomposite remediation technologies due to their ability to secrete large amount of enzymes, secrete large amount of proteins downstream processing and handling of the biomass would be much simple, economic viability, toleration and metal bioaccumulation capability, eco-friendliness, effective and fast growth (Moustafa, 2017).

Calcium oxide (CaO) is an exceptionally significant and important industrial compound, which is utilized as catalyst, toxic-waste treatment agent, water purification, an additive in refractory and in paint as well as for other major applications (El – Dawafry *et al*., 2015). Ultrafine metal oxide particles can be utilized as bactericide adsorbent. CaO has also shown great promise as a destructive adsorbent for toxic chemical materials. CaONPs are particularly interesting since it is considered safe substance for human and animals (Awaad *et al.*, 2024). The effectiveness of the prepared calcium oxide nanoparticles was achieved as an effective and inexpensive adsorbent for the removal of various heavy metal ions (iron, lead, manganese, chromium, and copper (II) from aqueous solutions, with a good opportunity to soon be integrated into technologies for treating polluted water and wastewater. CaONPs are a low-cost, versatile material utilized in a variety of environmental treatments (Awaad *et al*., 2024).

Furthermore, there are extensive studies on synthesis of calcium nanoparticles using plant and animal extracts but very little or no information on microbial synthesis and therefore require further studies to cover this research gap. Also, to the best of our knowledge, this is the first study of using multi – metal resistant endophytic fungi for biosorption application as well as using myco-synthesized calcium oxide nanoparticles for remediation of copper heavy metal polluted industrial effluents. This study was undertaken to investigate the potential of endophytic fungi and calcium oxide nanoparticles (CaONPs) for bioremediation and industrial wastewater treatment.

Materials and Methods

Material and sample collection

Fresh healthy Moringa seeds and leaves were purchased at Asaba market, Delta State, Nigeria. Pure calcium nitrate, reagents and other chemicals of analytical grade that were used in this study were purchased from Molychem and Loba Chem Companies, India representatives in Nigeria.

Identification of Plant Specimen

Prior to the experiments, the obtained Moringa seeds and leaves that were used for the isolation of the endophytic mould were undergo proper identification and deposition of plant at the Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State by Prof C.J. Ukpaka. A voucher specimen number were allocated to the plant in the herbarium of the Department (Iheukwumere *et al*., 2012a; 2012b; Mundi *et al*., 2013; 2014; Okoye *et al*. 2014; Anameze *et al*., 2023; Ezeamama *et al*., 2025a; 2025b; Afulukwe *et al.*, 2025; 2026; Umezulora *et al.*, 2026).

Isolation of Endophytic Fungi

By adopting the modified methods of Ebrahimi *et al*. (2010) and as described Okoye *et al*. (2020a); Uba *et al*. (2024); Mere *et al*. (2025) and Enemchukwu *et al*. (2026a); (2026b) the leaf and seed portions of the Moringa plant were thoroughly washed in running tap water, after which they were surface sterilized by submerging them in 75 % ethanol for 2 min. The portions were further sterilized sequentially in 5.3 % sodium hypochlorite solution for 5 min, and 75 % ethanol for 30 seconds. After drying, each leaf was divided into segments. For isolating endophytic fungi, 3 - 4 segments were placed on potato dextrose agar (PDA) supplemented with 50 mg/L

chloramphenicol to suppress bacterial growth. Then, the seed portions were cut to expose their inner tissue and placed on the same media. All the plates were incubated at 28 ± 2 °C for up to 4 - 7 days. The emerging fungi were transferred to fresh PDA plates, incubated at conditions above and periodically checked for purity. The predominant forms of fungal growth were tentatively selected and given a laboratory isolate number after purification.

Screening for the Metal Resistant Potential Strain

The isolates were screened for their potentials to tolerate multi - metals by adopting the modified methods of Manguilimotan and Bitacura (2018) and Dokubo *et al*. (2024). Initially, the fungal strains were grown on culture plates pre-filled with Potato Dextrose Agar (PDA) and incubated at 28 ± 2 °C for 7 days. Following incubation, mycelial agar plugs (6 mm^2) were cut approximately 5 mm from the colony margin and centrally inoculated on the surfaces of prepared sterile potato dextrose agar (PDA) plates containing increasing metal mixture concentrations of 25 ppm, 50 ppm, 75 ppm and 100 ppm. These concentrations fall in the range of the minimum inhibitory concentrations of some filamentous fungi on certain heavy metals. The plates without metal mixture served as the negative control. The plates were incubated at 28 ± 2 °C for 7 days. Presence or absence of growth were observed on the inoculated area. Minimum inhibitory concentration (MIC) were determined and defined as the lowest concentration of heavy metal/mL of medium that inhibited the visible growth of the test fungi. The colony with the most outstanding growth on the highest metal mixture concentration was considered as heavy metal tolerant fungal strain (Manguilimotan and Bitacura, 2018).

Characterization and Identification of the Most Tolerant Fungal Strain

The selected dominant and multi - metal resistant fungal strain was preliminary identified according to its macroscopic and microscopic characteristics. It was later identified molecularly to species levels for ITS rRNA genes identification using DNA extraction, gel electrophoresis, polymerase reaction, sequencing and blasting techniques (Moustafa, 2017; Manguilimotan and Bitacura, 2018).

Preparation of the Fungal Supernatant

For fungal biomass preparation, the selected most resistant fungal culture was grown in the 100 mL liquid medium containing (g/L): yeast, 3; peptone, 10; glucose, 20. Flask containing medium was incubated for 7 days at 28 ± 2 °C at 100 rpm. After the incubation, the biomass was harvested and washed with distilled water. Then, the biomass was added to 100 mL of deionized water and further incubated for 96 hr. After the incubation, the supernatant was obtained by passing the suspension through Whatman No. 1 filter paper and the filtrate (stock solution) was stored in the refrigerator (2 – 4 °C) for further work (Okoye *et al*. 2020b; 2020c).

Synthesis of Calcium Oxide Nanoparticle Using Fungal Filtrate

In this test, 50 mL fungus filtrate which serves as reducing agent was boiled at 40 – 50 °C using a magnetic stirrer. The temperature of the solution was reached at 40 – 50 °C; 5 g of calcium nitrate tetrahydrate ($Ca(NO_3)_2\,H_2O$) in 100 mL distilled water which serves as Ca precursor was added in dropwise addition under magnetic stirrer for 2 hrs. The pH of the reaction mixture was adjusted to 12 using 2 M NaOH to facilitate particle formation. A change in solution color was observed, which indicates the reduction of Ca^{2+} ions and the formation of $Ca(OH)_2$NPs (Gamal et al., 2024). The solution was filtered through filter paper, and the precipitate was transferred to a crucible and heated in a muffle furnace up to 400 °C for 3 hrs to obtain the white powder (Jadhav et al., 2022).

Physical and Chemical Characterization of Calcium Oxide Nanoparticles

The produced Calcium Oxide Nanoparticle was characterized by visible colour change determination, UV-Visible spectroscopy at the wavelengths ranging from 200 - 800 nm. The phase purity and crystalline nature was determined by X - ray Diffractometer. The size, morphology and elemental composition of the formed NPs was determined by using scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS). The Fourier transform infrared (FTIR) spectroscopy was used to determine the functional groups of produced NPs (Dokubo and Uba, 2023; Uba and Obiefuna, 2023; Okafor *et al*., 2023; Ubani *et al*., 2024a; 2024b; 2025; Ekwenze *et al*., 2025; Ele *et al*., 2025; Uba and Okonkwo *et al*. 2025; Okwonkwo *et al.* 2026 and Uba *et al.* 2026a).

Collection of Industrial Effluent Sample

The effluent samples were collected from two industrial areas as follows: textile industrial waste water designated as sample A and leather industrial wastewater from Lagos State Industrial Leather Hub, situated in the **Matori Industrial Estate, Mushin** designated as sample B . All the samples were collected randomly at different points with well labeled sterile sampled 2 L bottles and later mixed together as composite samples (Alfred *et al*. 2023; 2025; Idu *et al*., 2026a; 2026b; Ibo *et al*. 2020; Ibe *et al*. 2023, Chukwura *et al*. 2025; Uba and Udaba *et al*. 2026; Uba *et al.* (2026a).

Determination of Physicochemical Parameter on Industrial Effluent Sample

The temperature, pH, conductivity, total dissolved solids, turbidity, DO, BOD_5, COD, nitrate, phosphate and sulphate of the prepared effluent samples were determined by adopting the standard method of APHA (2012) and as described by Uba, (2019); Nwigwe *et al*. (2022), Nwigwe *et al*. (2023), Ifediegwu *et al*. (2023a); (2023b); Ifediegwu *et al. (*2024a), (2024b); (2024c), Orji and Oghonim *et al.* (2023) and Nnaka *et al*. (2024).

Determination of Heavy Metal Content of the Industrial Effluent Sample

The heavy metal content (copper, nickel, lead, zinc, mercury, arsenic, chromium, cadmium) of the industrial effluent samples from the different industrial areas were analyzed using atomic absorption spectrophotometer by after acid digestion adopting the method of APHA (2012**)** and as described by Uba *et al*. (2020c); (2020d); (2020e); (2020f); (2020g) and (2020h)**.**

Biosorbent Preparation

The biomass of the most tolerant test fungus was prepared in a yeast peptone glucose liquid medium (YPG). After harvesting by centrifugation at 5,000 rpm for 20 min and subsequent filtration with 0.45 µm filter, washing three times with distilled water and the biomass was dried in on hot air oven at 60 °C for 24 hr, powdered using mortar and pestle and was utilized in further biosorption studies (Okafor *et al*. 2021a; 2021b; Ofunwa *et al*. 2024).

Optimization and Metal Biosorption Studies of Industrial Wastewater by the Test Fungus and Calcium Oxide Nanoparticle

Laboratory experiment was performed on five periods (5, 25, 60, 120, 240 min), five pH ranges (3, 5, 7, 9, 12), four temperature ranges (20, 30, 40, 50 °C) and five doses of adsorbent blank and calcium oxide nanoparticles (0.1, 0.5, 1.0, 2.0, 3.0 g) per 100 mL industrial effluent samples). Then, the metal ion solutions in the industrial effluent samples contact with the biosorbent and adsorbent were shaken at 150 rpm rotary shaker while keeping the values of other parameters same as stated above in duplicate (Sravanthi *et al.*, 2018) and the equilibrium concentration of each metal was determined). Biosorption capacity was calculated using the mass balance formula below:

$$\mathrm{qe} = \frac{(C_O - C_t)V}{m}$$

Where q_e (mg/g) is the amount of metals adsorbed onto the test fungus, calcium oxide nanoparticles, respectively; C_0 and C_t were the initial and final concentration of metals in the solution, respectively; m (g) is the mass of the test fungus, calcium oxide nanoparticle and V (mL) is the volume of the liquid phase.

Also, the removal percentage (R %) of each metal will be calculated for each run by using the equation:

$$R(\%) = \left(1 - \frac{C_t}{C_o}\right) x100$$

Statistical Analysis

All assays were conducted in duplicates and triplicates and mean ± standard deviation was calculated. All experimental data were analyzed with GraphPad Prism version 9.0 using the ANOVA test with Dunnett's multiple comparison test. A *p*-value less than 0.05 was considered as statistically significant at 95 % confidence interval (Uba and Chukwura, 2016; Okeke *et al*. 2025a; 2025b; Afulukwe *et al.*, 2025; 2026).

Results

The results of the tolerance levels of the endophytic strains to various heavy metals in Table 1. From the Table 1, strain 1 demonstrated broad-spectrum tolerance across all metals and concentrations. Notably, mercury tolerance was highest at 25 ppm (7.50), decreasing with increasing concentration; lead tolerance increased significantly at 100 ppm (6.30); cadmium tolerance remained relatively stable across concentrations and arsenic tolerance remained comparatively low but present at all levels. The relatively high tolerance to Hg and Pb suggested strong detoxification mechanisms, potentially involving intracellular sequestration or extracellular binding to cell wall components. The decreasing Hg tolerance at higher concentrations may indicate threshold toxicity beyond which metabolic systems are overwhelmed.

Strain 2 showed variable but notable tolerance patterns with copper tolerance increased progressively, peaking at 100 ppm (4.30); cadmium tolerance was highest at 50 ppm (4.00); arsenic tolerance was absent at 25 and 75 ppm but highest at 100 ppm (6.30) and mercury tolerance fluctuated but remained moderate. The marked increase in arsenic tolerance at 100 ppm suggests inducible resistance mechanisms, possibly involving activation of arsenic efflux pumps or reduction pathways.

Strain 3 exhibited selective tolerance with strong tolerance to Hg across all concentrations, peaking at 100 ppm (5.30); moderate Cd tolerance at 25 and 50 ppm and complete inhibition (0.00) at higher Cu, Pb, Cd, and As concentrations. This pattern suggests specialization toward mercury resistance, possibly through mercuric reductase activity or sequestration mechanisms. The absence of growth under several metals at 75 and 100 ppm indicates limited adaptability compared to other isolates.

Strain 4 demonstrated remarkable cadmium tolerance at 75 ppm (8.10), the highest single value recorded in the table. Additional observations include increasing arsenic tolerance with concentration, reaching 5.80 at 100 ppm; moderate copper and lead tolerance and minimal mercury tolerance except at 50 ppm. The exceptional Cd tolerance suggests efficient metal-binding capacity, possibly mediated by chitin-rich cell walls, extracellular polymeric substances, or metallothionein production.

Strain 5 showed moderate tolerance to Pb at 75 ppm (5.90) and increasing arsenic tolerance at 100 ppm (5.10). However, copper tolerance declined with increasing concentration; mercury tolerance remained generally low and cadmium tolerance decreased at higher concentrations. This pattern indicates partial resistance, with stronger adaptation to Pb and As than to Cu or Cd.

Table 1: Tolerance levels of the endophytic strains to various heavy metals

Organism	Concentration (ppm)	Copper (Cu)	Lead (Pb)	Cadmium (Cd)	Mercury (Hg)	Arsenic (As)
	25	3.60	3.10	5.00	7.50	1.00
1	50	4.10	2.90	3.10	4.80	2.00
	75	4.30	4.00	3.50	3.80	2.50
	100	3.30	6.30	4.80	4.30	2.30
	25	1.70	2.90	2.60	2.40	0.00
2	50	2.90	3.20	4.00	1.90	3.80
	75	3.30	2.70	3.90	4.70	0.00
	100	4.30	3.40	0.00	3.70	6.30
	25	1.80	0.00	4.40	4.60	1.00
3	50	3.10	1.30	3.90	4.90	0.00
	75	0.00	4.40	2.00	2.90	0.00
	100	0.00	0.00	0.00	5.30	0.00
	25	3.10	2.20	2.50	0.00	0.00
4	50	0.00	1.50	2.30	2.20	4.10
	75	1.90	2.00	8.10	0.00	4.00
	100	3.70	3.40	2.00	0.60	5.80
	25	3.50	0.00	1.60	1.50	0.00
5	50	2.40	2.50	2.00	0.00	1.60
	75	1.90	5.90	1.50	1.90	1.90
	100	0.00	0.00	0.90	1.10	5.10

Table 2 presented the macroscopic and microscopic characteristics of five endophytic fungal isolates recovered and identified based on standard mycological criteria. The identification relied on colony morphology (surface texture, pigmentation, growth pattern, and reverse colouration) in conjunction with microscopic features such as hyphal structure, conidial morphology, spore arrangement, and specialized reproductive structures. The integration of these phenotypic traits enabled the tentative classification of the isolates into five distinct fungal taxa: *Curvularia lunata*, *Aspergillus flavus*, *Alternaria alternata*, *Fusarium solani*, and *Rhizopus oryzae*.

Table 2: Morphological and microscopic identification of the endophytic strains

Isolates	Macroscopic features	Microscopic features	Probable organism
1	Colonies were grey but turned greyish black as they age	Large bent spores with 3-5 cells similar to *Helminthosporium* spp., brown septate hyphae	*Curvularia lunata*
2	Colonies were white but later turned black, the reverse side was pale yellow	Septate hyphae, conidiospores bearing conidial heads containing conidia were seen	*Aspergillus flavus*
3	Aerial mycelium, dark green, beige reverse	Articulated conidiospores bearing dense spores arranged in twos, spores are brown and spherical in shape	*Alternaria alternata*
4	Disc shape, white at first and then grown to a cloudy surface with cream mycelium (non-pigmented bottom)	Broad hyphae with many terminal clamydospores with some oval microconidia, string bean shaped conidia and clamydospores are in chain	*Fusarium solani*
5	White colony mycelium with black dots and covers the entire plate	Sporangiospores are produced inside a spherical sporangium, columella is present on the tip of the sporangiospore, root-like rhizoids are seen	*Rhizopus oryzae*

The UV - VIS spectral profile of *Curvularia lunata* biosynthesized calcium oxide nanoparticle is shown in Figure 1. Figure 2 showed the Infra-red spectral profile of *Curvularia lunata* biosynthesized calcium oxide nanoparticle. Figure 3 showed the X ray diffraction profile of *Curvularia lunata* biosynthesized calcium oxide nanoparticle. Figure 4 showed the scanning electron micrograph profile of *Curvularia lunata* biosynthesized calcium oxide nanoparticle. Figure 5 showed the Gamma-ray energy spectrum profile of *Curvularia lunata* biosynthesized calcium oxide nanoparticle. Table 3 showed the elemental composition and distribution of *Curvularia lunata* biosynthesized calcium oxide nanoparticle.

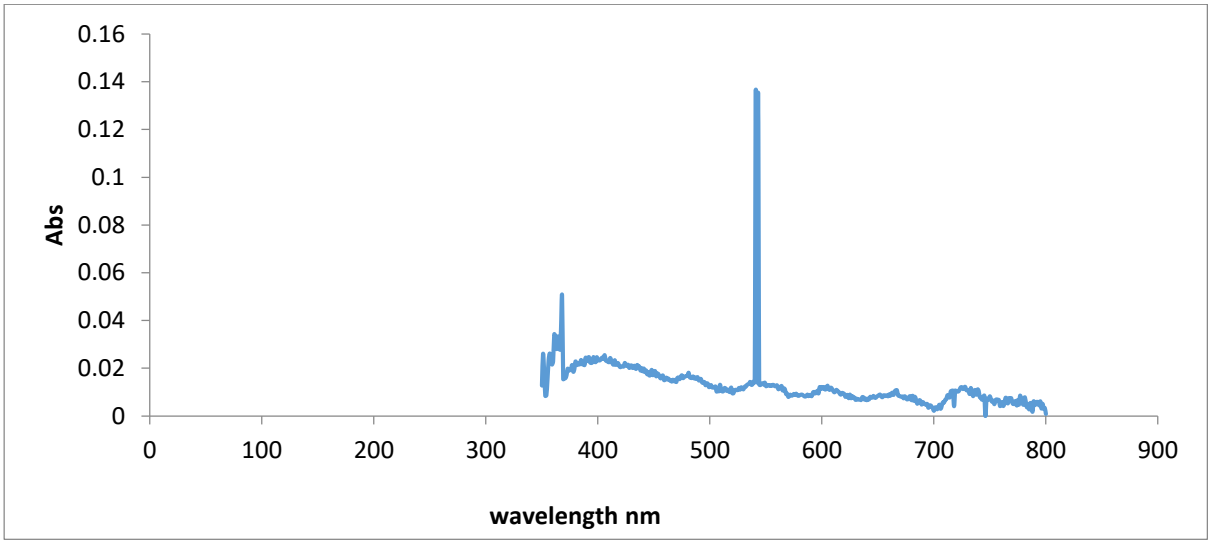

Figure 1: UV - VIS spectral profile of *Curvularia lunata* biosynthesized calcium oxide nanoparticle

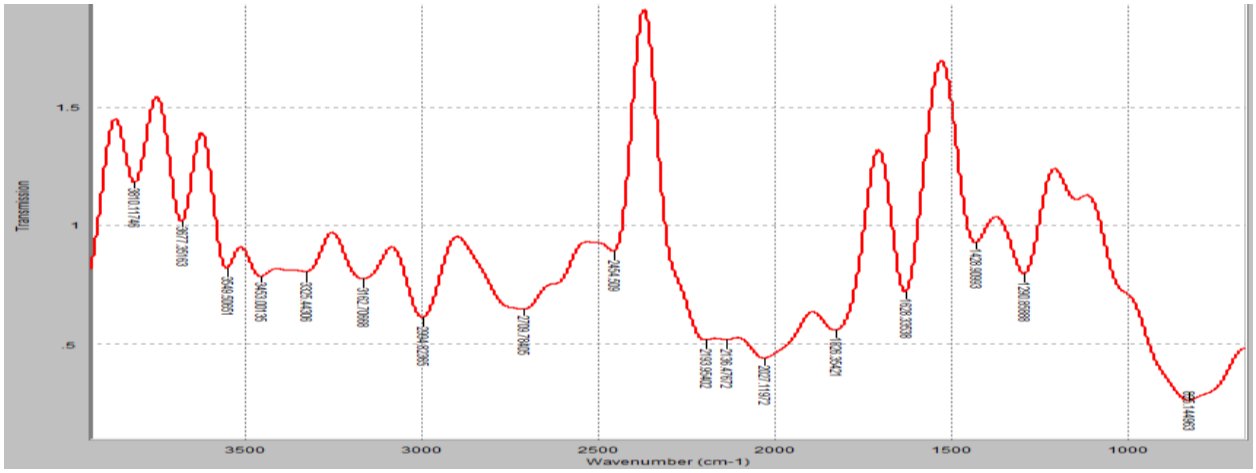

Figure 2: Infra-red spectral profile of *Curvularia lunata* biosynthesized calcium oxide nanoparticle

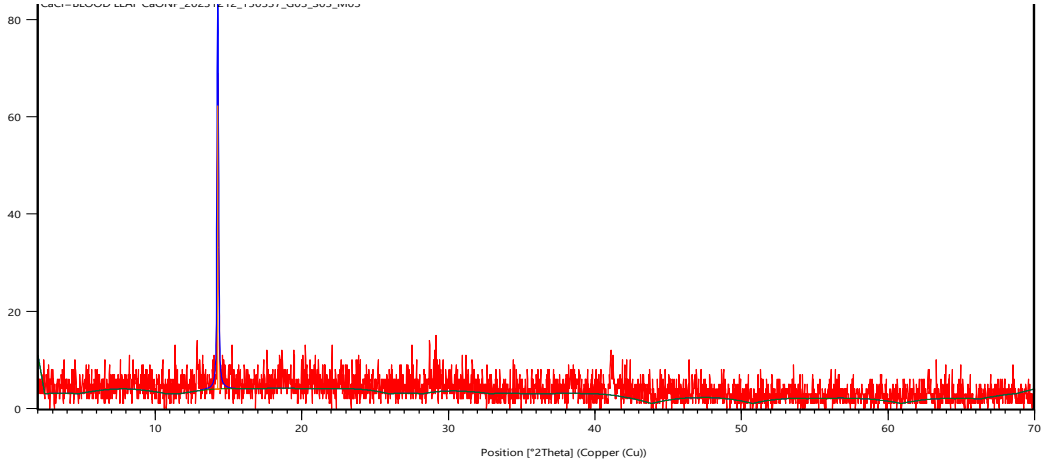

Figure 3: X-ray diffraction profile of *Curvularia lunata* biosynthesized calcium oxide nanoparticle

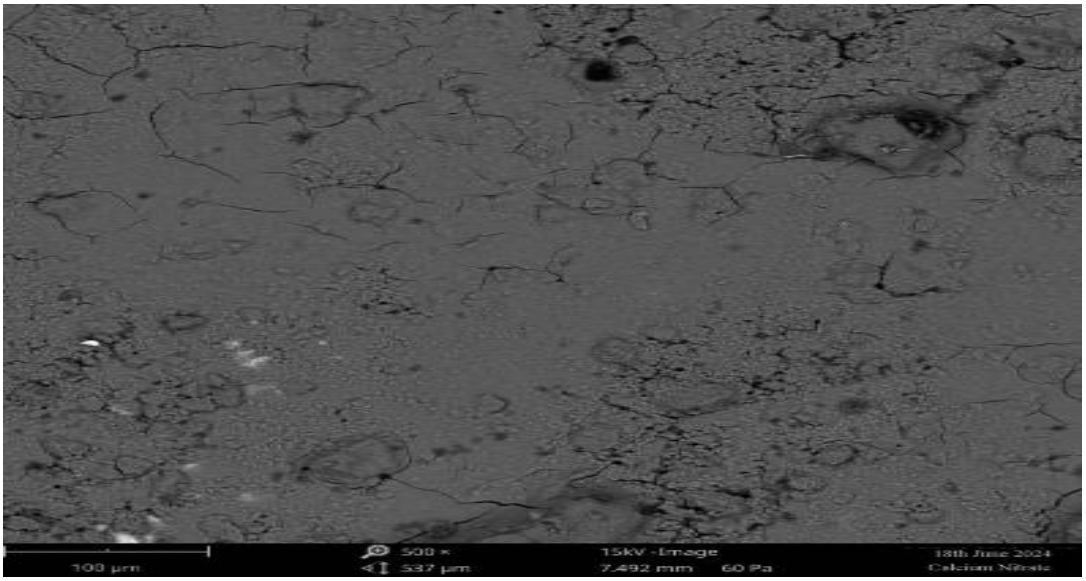

Figure 4: Scanning electron micrograph profile of *Curvularia lunata* biosynthesized calcium oxide nanoparticle

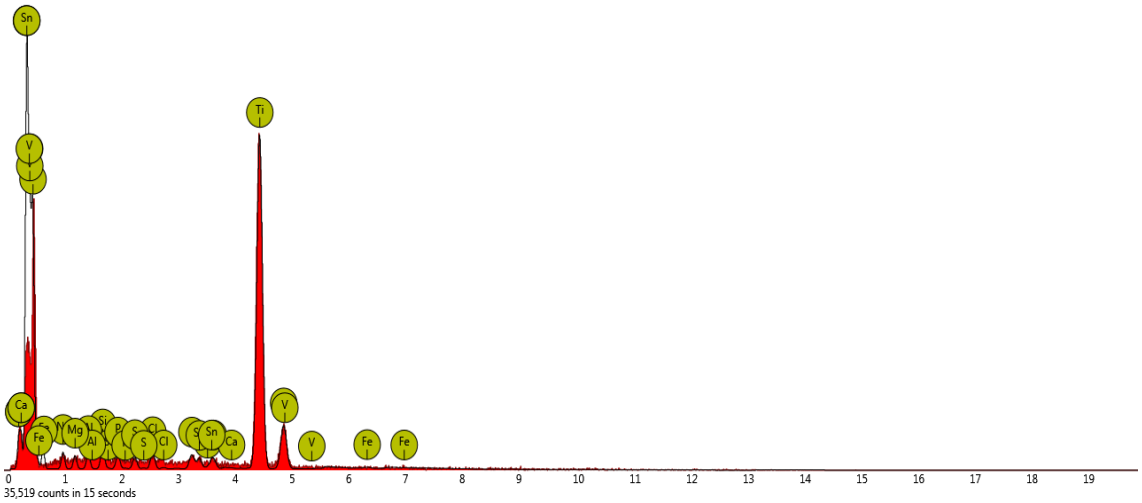

Figure 5: Gamma-ray energy spectrum profile of *Curvularia lunata* biosynthesized calcium oxide nanoparticle

Table 3: Elemental composition and distribution of *Curvularia lunata* biosynthesized calcium oxide nanoparticle

Element number	Element symbol	Element name	Atomic conc.	Weight conc.
20	Ca	Calcium	52.34	58.74
11	Na	Sodium	7.33	8.83
50	Sn	Tin	1.41	3.81
19	K	Potassium	7.18	6.94
12	Mg	Magnesium	8.40	6.88
14	Si	Silicon	2.85	1.82
17	Cl	Chlorine	2.14	1.72
22	Ti	Titanium	11.87	1.71
13	Al	Aluminium	2.73	1.67
15	P	Phosphorus	1.92	1.35
16	S	Sulfur	1.48	1.08
26	Fe	Iron	0.34	0.44
23	V	Vanadium	0.00	0.00

The temperature of the textile wastewater is 30.30°C, slightly higher than the leather wastewater at 28.50°C, but both fall within the typical ≤30–35°C range accepted for industrial effluent discharge (Table 4). Textile wastewater has a near-neutral pH of 6.80, while leather wastewater is very alkaline (9.95). The WHO/standard recommended range is 6.5 – 8.5. Textile wastewater exhibits high conductivity (1325.10 µS/cm) and TDS (1330 mg/L) exceeding the standard ≤1000 limit. Leather wastewater shows much lower values (30.78 µS/cm and 26 mg/L). Textile wastewater turbidity is 190 NTU, considerably higher than leather wastewater (2 NTU) and the standard ≤5 NTU limit. Both wastewater types have DO below the standard ≥5 mg/L, with textile at 4.15 mg/L and leather at 4.32 mg/L. Textile wastewater shows BOD_5 of 21 mg/L and COD of 91 mg/L, within the typical ≤30–50 and ≤100–250 limits. Leather wastewater is very low (BOD_5 2.16; COD 4.90). Textile wastewater nitrate is 0.15 mg/L, leather is 0.65 mg/L, both well below the standard ≤50 mg/**L.** Textile wastewater phosphate (6.23 mg/L) slightly exceeds the standard ≤5 mg/L, while leather wastewater (0.91 mg/L) is within limits. Textile wastewater sulphate (89.30 mg/L) is below the standard ≤500 mg/L, while leather wastewater has low sulphate (2.00 mg/L).

Table 4: Baseline physicochemical profile

Parameter	Textile Wastewater	Leather Wastewater	WHO/Standard Limits
Temperature (°C)	30.30	28.50	≤ 30–35°C
pH	6.80	9.95	6.5 – 8.5
Conductivity (µS/cm)	1325.10	30.78	≤ 1000
Total Dissolved Solids (mg/L)	1330.00	26.00	≤ 1000
Turbidity (NTU)	190.00	2.00	≤ 5
Dissolved Oxygen (DO) (mg/L)	4.15	4.32	≥ 5 mg/L
BOD_5 (mg/L)	21.00	2.16	≤ 30 – 50
COD (mg/L)	91.00	4.90	≤ 100 – 250
Nitrate (mg/L)	0.15	0.65	≤ 50
Phosphate (mg/L)	6.23	0.91	≤ 5
Sulphate (mg/L)	89.30	2.00	≤ 500

The Figure 6 showed the removal performance of untreated (control) and calcium oxide nanoparticle (CaONP)–treated textile and leather wastewater over 240 minutes. Control samples exhibited minimal removal (<15 %) throughout the timeframe. In contrast, CaONP treatment substantially enhanced removal efficiency. For textile wastewater, removal increased rapidly from 30 % at 5 minutes to a peak of 91 % at 60 minutes, then declined to 58 % at 240 minutes. Leather wastewater treated with CaONPs showed a similar trend, increasing from 22 % at 5 minutes to 78 % at 60 minutes and peaking at 85 % at 120 minutes, followed by a decrease to 50 % at 240 minutes. Overall, CaONP treatment markedly outperformed the untreated controls, with maximum efficiency observed between 60 and 120 minutes.

The Figure 7 showed the response of control and CaONP-treated textile and leather wastewater across pH levels 3, 5, 7, 9, and 12: Both treated and control samples show peak performance at neutral pH (7), with CaONP-treated effluents significantly higher than controls across all pH levels. Performance drops substantially toward both acidic (pH 3) and highly alkaline (pH 12) extremes. Treated textile and leather wastewater show nearly doubling or greater increase**s** in measured values at pH 7 compared to controls, implying a strong effect of CaONPs near neutral conditions.

The Figure 8 showed the effect of temperature on control and CaONP-treated textile and leather wastewater. Both control textile and leather wastewater show an increase from 20 °C to 30 °C, with control textile rising from 4 to 40 % and leather from 2 to 34 %. After 30 °C, control responses decline at 40 °C and further at 50 °C. Meanwhile, both treated textile and leather wastewater peaked at 30 °C (87 % and 82 %, respectively). After 30 °C, treated responses decrease at 40 °C and further at 50 °C but remain above controls at each temperature. Maximum treatment effectiveness is observed at 30 °C, with CaONP-treated samples consistently outperforming controls across all temperatures.

The Figure 9 showed responses of control and CaONP-treated textile and leather wastewater across different concentration levels (treatment dosage). For both control textile and leather wastewater, control responses increased from 0.1 to 0.5 g/ 100 mL, then plateaued or slightly decreased at higher levels. Textile control peaked at 13 % (0.5 g/ 100 mL), while leather control peaked at 8 % (1 g/ 100 mL). Also, the treatment with CaO nanoparticles markedly increased responses compared to controls at all levels. For textile wastewater, the highest response was at 1 g/ 100 mL (91 %), followed by 0.5 g/ 100 mL (82 %) and 2 (85), with decreasing values at very low (0.1 g/ 100 mL) and higher (3 g/ 100 mL) levels, respectively. For leather wastewater, the highest treated value was also at 2 g/ 100 mL (86 %) and 1 g/ 100 mL (80 %), with lower responses at the extremes (0.1 and 3 g/ 100 mL). CaONP treatment significantly improved wastewater response metrics relative to controls across all levels, with peak effectiveness observed around moderate levels (0.5 – 2 g/ 100 mL**)** and reduced responses at extremes.

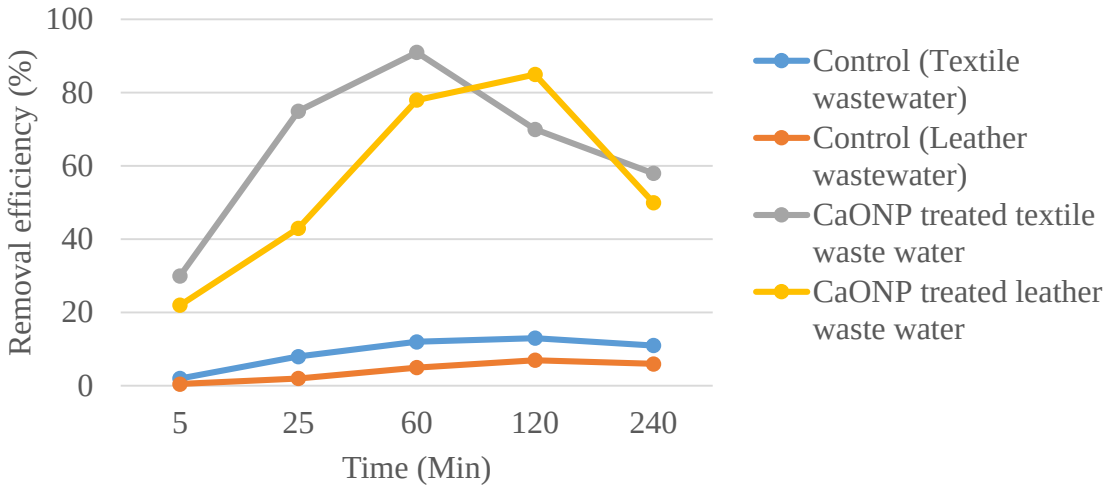

Figure 6: Effect of time on waste water copper metal removal efficiency by *Curvularia lunata* biosynthesized calcium oxide nanoparticle.

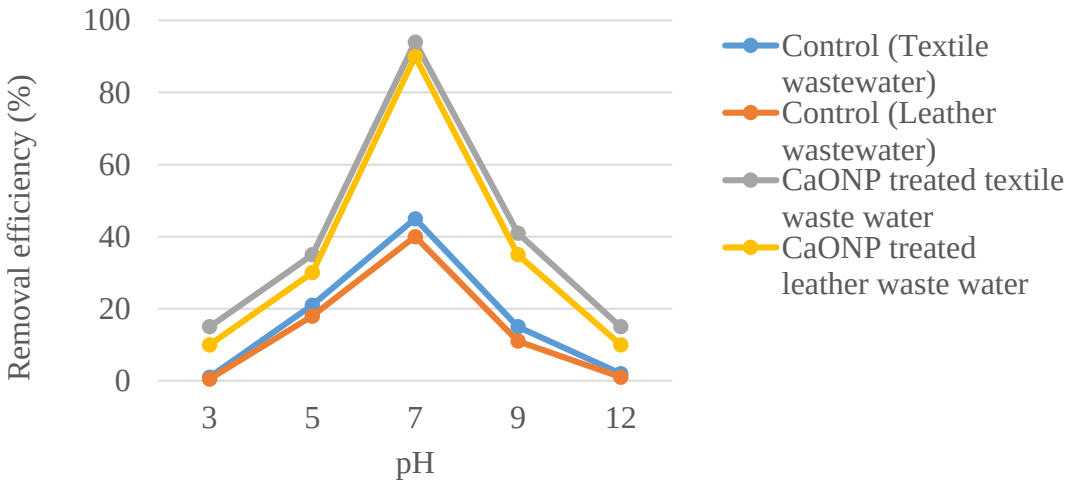

Figure 7: Effect of pH on waste water copper metal removal efficiency by *Curvularia lunata* biosynthesized calcium oxide nanoparticle

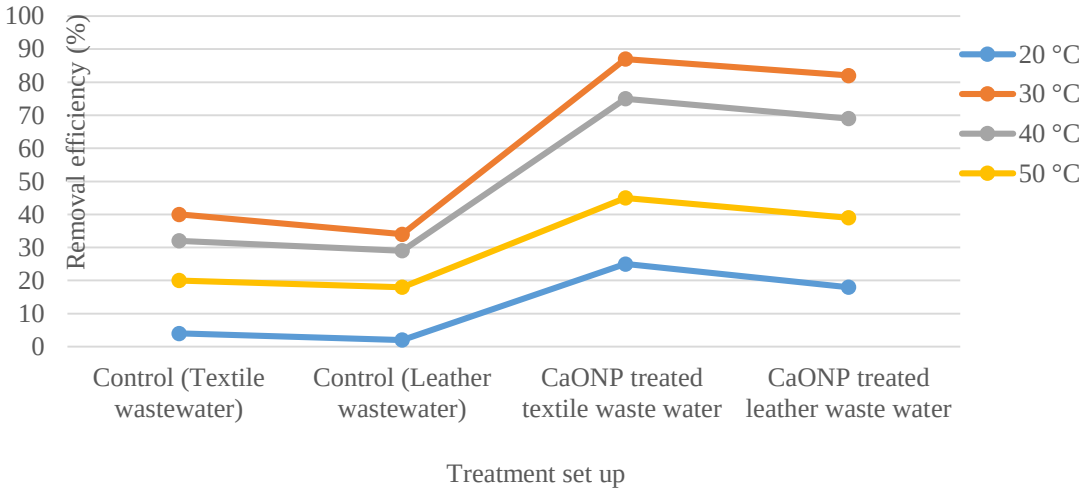

Figure 8: Effect of temperature on waste water copper metal removal efficiency by *Curvularia lunata* biosynthesized calcium oxide nanoparticle

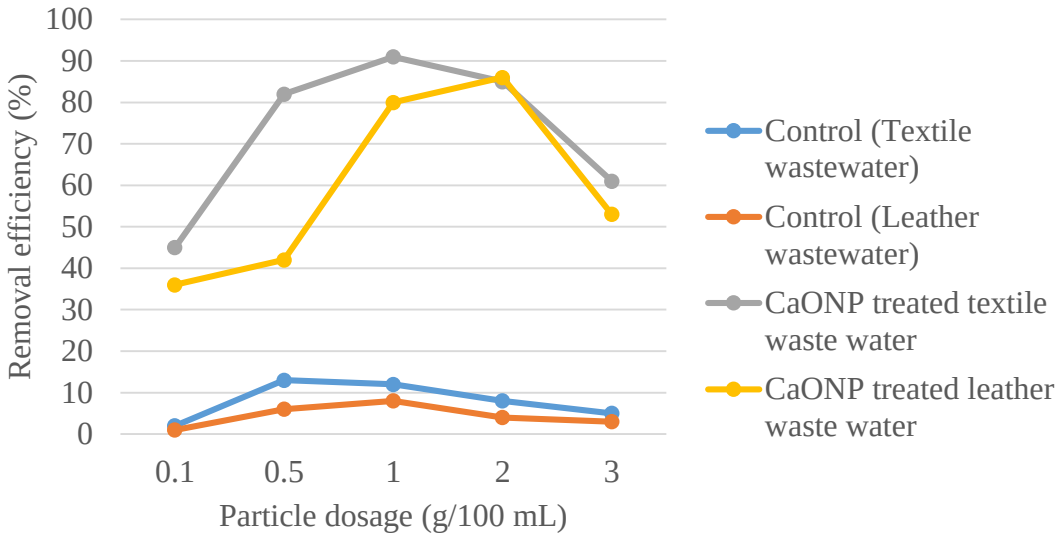

Figure 9: Effect of particle dosage on waste water copper metal removal efficiency by *Curvularia lunata* biosynthesized calcium oxide nanoparticle

Discussion

Table 1 presented the tolerance profiles of five endophytic fungal strains exposed to increasing concentrations (25 – 100 ppm) of five heavy metals: copper (Cu), lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As). The tolerance values (expressed as growth indices) indicate differential responses among isolates, reflecting species-specific resistance mechanisms and adaptive capacities. Heavy metal tolerance in endophytes is widely associated with metal sequestration, biosorption, intracellular compartmentalization, enzymatic detoxification, and antioxidant defense systems (Kumar *et al*., 2023; Rai *et al*., 2022). Across all isolates, mercury (Hg) and cadmium (Cd) showed relatively higher tolerance values at certain concentrations, whereas arsenic (As) frequently resulted in zero growth in several isolates, particularly at lower concentrations. This pattern may reflect differences in metal bioavailability, ionic toxicity, and the metabolic cost of detoxification. Copper (Cu) and lead (Pb) exhibited moderate to high tolerance in most isolates, particularly at 75 and 100 ppm, suggesting effective detoxification or sequestration systems. Fungal tolerance to Cu and Pb has been linked to metallothionein production, melanin binding, and active efflux systems (Rai *et al*., 2022). The observed variability underscores the ecological adaptability of endophytic fungi in metal-contaminated environments. Endophytes inhabiting stressed environments often develop enhanced detoxification systems that may contribute to host plant survival under heavy metal stress (Domka *et al*., 2019). Overall, the data reveal considerable variability both among metals and across concentrations, suggesting that tolerance is not only species-dependent but also metal-specific and dose-dependent.

The results presented in Table 2 demonstrate considerable morphological diversity among the isolated endophytic fungi. Three isolates (*C. lunata*, *A. alternata*, and *A. flavus*) belong to the Ascomycota and exhibit septate hyphae and conidial reproduction. *F. solani* similarly belongs to the Ascomycota but is distinguished by its macroconidial morphology and chlamydospore formation. In contrast, *R. oryzae* represents a member of the Mucorales, characterized by aseptate hyphae and sporangial reproduction. The identification process highlights the importance of combining macroscopic colony characteristics with detailed microscopic observations. Colony pigmentation, growth rate, and texture provide preliminary clues, while spore morphology, septation, and reproductive structures offer definitive taxonomic evidence These endophytic strains have been isolated and documented by several authors (Amaike & Keller, 2011; Walther *et al*., 2013; Woudenberg *et al*., 2015; Marin-Felix *et al*., 2017; Samson *et al*., 2014; Samson *et al*., 2019; Nilsson *et al*., 2019). Overall, the diversity of fungal genera identified suggests a complex endophytic community within the host plant. Such diversity may have ecological and biotechnological implications, as endophytes are known to contribute to plant health, stress tolerance, and the production of secondary metabolites. The findings therefore provide a foundational basis for further molecular confirmation and functional characterization of the isolates.

The strain 1 which was found to be the most potent metal resistant fungal strain out of the five selected fungal strains and later identified as *C. lunata* was used for the biosynthesis of CaONPs. The produced particles were physicochemically characterized and the result in Figure 1 presented the UV–visible spectrum, with wavelength plotted on the horizontal axis and absorbance on the vertical axis. The calcium oxide nanoparticles (CaONPs) synthesized by *Curvularia lunata* exhibited a maximum absorbance of 0.0145 at 539 nm. This absorption peak suggests the presence of bioactive compounds such as phenolic acids, flavones, flavonols, and other flavonoids capping the surface of the nanoparticles (Mazher *et al*., 2023). Figure 2 illustrates the FTIR spectrum of the same

biosynthesized CaONPs, showing wavenumber on the x-axis and peak intensity on the y-axis. The FTIR results confirm that phytochemicals were adsorbed onto the nanoparticle surface, contributing to their enhanced stability. Characteristic vibrational bands were observed at 3639 cm^{-1} (alcohols or phenols), 2860 cm^{-1} (carboxylic acids and aldehydes), 2487 cm^{-1} (alkynes), 1625 cm^{-1} (amines), and 1434 cm^{-1} (alkanes), indicating the involvement of various functional groups in nanoparticle stabilization.

Figure 3 displayed the X-ray diffraction (XRD) pattern, with theta values on the horizontal axis and electron count intensity on the vertical axis. The diffractogram revealed prominent peaks at higher theta values for the *Curvularia lunata*–mediated CaONPs, confirming their crystalline nature. The XRD technique operates on the principle that incident X-rays interact with electrons in the atomic structure of the nanoparticles, causing scattering. These scattered rays form interference patterns; however, only constructive interference produces observable diffraction peaks (Fultz & Howe, 2013). Because nanoparticles possess a high surface area-to-volume ratio, their physicochemical properties vary significantly with size, making detailed characterization essential. Factors such as surface texture, strain, particle shape, crystalline phase, defects, and crystal size strongly influence their chemical, electronic, mechanical, and optical behavior. Without comprehensive characterization, determining the suitability and application potential of specific nanoparticles becomes highly challenging (Thanh *et al*., 2014).

The result in Figure 4 scanning electron microscopy (SEM) image revealed that the particles have an irregular shape with a rough surface and fractured texture. Additionally, the particles tend to have a spherical shape and possess a porous structure with numerous cavities, similar to a porous material. This porous structure is believed to contribute to the increased presence of basic sites on the catalyst and corroborated with finding of Chukwuemeke *et al*. (2024). The Figure 5 and Table 3 results revealed that the sample is rich in calcium, with moderate amounts of titanium, magnesium, sodium, potassium and trace levels of other elements and corroborated with the finding of Shyamala *et al*. (2020).

Physicochemical indices are important markers for determining the quality of waste water sample and the result in Table 4 demonstrated the baseline profiles of textile and leather wastewater samples. Elevated wastewater temperature is often associated with heat released during dyeing, washing, and finishing processes in the textile industry (Oluyemi *et al*., 2024). Leather processing also generates thermal wastewater due to chemical treatments and soaking steps (El-Baky & El-Shahat, 2023). While both values are within acceptable limits, temperatures near the upper limit can reduce dissolved oxygen levels in receiving waters, negatively affecting aquatic life (Zalina *et al*., 2021). Textile effluents commonly contain acidic or alkaline residues depending on the dyes and auxiliaries used (Kumar & Saini, 2023). The leather wastewater's high pH reflects extensive use of lime and chrome in the tanning process (Mishra & Kalita, 2022). Alkaline wastewater outside the guideline range can increase toxicity, reduce metal bioavailability, and disrupt aquatic ecosystems (Deng *et al*., 2023). High conductivity and TDS in textile wastewater indicate significant dissolved salts from dyes, detergents, and finishing chemicals (Upadhyay *et al*., 2024). Elevated TDS can impair water taste and hinder aquatic life due to osmotic stress (Salih & Almuktar, 2023). Conversely, the low values for leather wastewater may result from on-site dilution or treatment prior to sampling. High turbidity in textile effluent is attributed to suspended solids from fibers, dyes, and process residues (Singh et al., 2023). Turbidity reduces light penetration, affecting primary production and aquatic food webs (Wu & Huang, 2022). Low turbidity in the leather effluent suggests effective preliminary solids removal or lower suspended load. Low DO levels in industrial wastewater often arise from high organic load and microbial oxygen consumption (Zhang *et al*., 2024). Reduced DO can stress aquatic organisms and impair self-purification capacity of receiving waters (Li *et al*., 2023). This calls for enhanced treatment to raise DO before discharge. These values reflect moderate organic pollution, with textile effluents containing more biodegradable organics than leather wastewater (Raza *et al*., 2024). High BOD/COD ratios commonly indicate readily biodegradable organics from detergents and dyes, influencing treatment design (Parker & O'Connor, 2023). Low nitrate suggests minimal nitrogenous pollution, likely due to absence of significant fertilizer inputs or effective nitrogen removal (Smith & Choi, 2022). These low concentrations pose minimal direct eutrophication risk. Phosphates in textile wastewater originate from detergents and processing aids (Ahmed *et al*., 2023). Elevated phosphate contributes to eutrophication in surface waters, encouraging algal blooms (Nguyen & Yang, 2023), so removal is often required. Sulphate originates from dyes and auxiliary chemicals in textile processes (Rafiq *et al*., 2024). While within acceptable discharge limits, high sulphate can contribute to odor problems and, under anaerobic conditions, lead to hydrogen sulphide formation (Cheng & Lim, 2023).

The results revealed that textile wastewater generally exhibits higher pollutant loads—especially in conductivity, TDS, turbidity, and phosphate—compared with leather wastewater, likely due to the wide range of chemicals used in textile processing. Both effluent types show parameters outside recommended limits for pH, DO, and turbidity, suggesting the need for improved on-site treatment before discharge into the environment. Industrial wastewater with high salinity, low oxygen, and nutrient imbalances can severely impact aquatic ecosystems, highlighting the importance of strict monitoring and treatment to comply with WHO-based standards and protect water quality.

Industrial effluents from the textile and leather sectors contain high loads of organic dyes, heavy metals, and complex organic compounds that pose ecological and health threats if released untreated (Rezania *et al*., 2024). Traditional treatment methods such as coagulation, biological oxidation, and

advanced oxidation processes often struggle with efficiency, cost, and sludge generation. Metal-oxide nanoparticles, particularly calcium oxide nanoparticles (CaONPs)**,** are increasingly studied for their high surface area, reactivity, and adsorption capabilities in water purification applications. Nanoparticles can enhance removal of organic pollutants and metals via adsorption, precipitation, and catalysis where conventional methods lag behind (Zia *et al.,* 2025).

The results in Figure 6 indicated that CaONP treatment significantly enhances metal removal compared with untreated wastewater. The untreated (control) samples showed negligible removal across all time points, which is consistent with prior studies demonstrating poor self-remediation in industrial effluents without active treatment (Patil & Gaware, 2022). This confirms that raw textile and leather wastewater require targeted remediation strategies to reduce heavy metal contamination effectively. The differing profiles between textile and leather wastewater further emphasize matrix complexity influences. Leather wastewater often contains additional interfering species (e.g., chromium and organic compounds) that can compete for adsorption sites and modify removal dynamics (Reddy & Venkataramani, 2023). This results in variations in peak removal timing and efficiency, underscoring the need to tailor treatment strategies to specific wastewater compositions. Mechanistically, CaONP performance is explained by adsorption, precipitation, and surface complexation interactions, which facilitate heavy metal binding and removal. The presence of calcium hydroxide groups and high surface area enhances interaction with metal ions, as described in recent nanoparticle remediation literature (Siddiqui & Khan, 2024).

The result in Figure 7 indicated that both textile and leather wastewater responded strongly to CaO nanoparticle (CaONP) treatment at pH 7**,** with treatment values more than double the control at this point. Such behavior aligned with general trends in nanoparticle-based wastewater treatment, where neutral or near-neutral pH favoured pollutant adsorption and degradation, due to optimal surface charge interactions and reduced solubility constraints of contaminants. Nanoparticle surface chemistry and pollutant ionization states are often most compatible at neutral pH, enhancing removal efficiency. At extreme pH (3 or 12), the effectiveness dropped significantly for both textile and leather wastewaters. Very acidic or very alkaline conditions can diminish interaction between the nanoparticle surface and pollutants by either protonating or deprotonating active sites, which reduces electrostatic attraction and adsorption capacity. Similar pH dependencies were noted in nanomaterial adsorption studies, where performance is greatest near the point of zero charge (pHpzc) and lowers at distant pH values (Rezania *et al*., 2024). The control values for leather wastewater were generally lower than textile across all pH levels, suggesting that untreated leather wastewater initially contains fewer reactive or measurable components under the employed metric. After CaONP treatment, both wastewater types exhibited improvements, but the textile wastewater saw a slightly higher absolute increase in treatment response—especially at pH 7 (treated textile: 94 %vs treated leather: 90 %). This pattern may reflect differences in the composition and pollutant load of textile vs. leather wastewater. Textile effluents commonly contain dyes, surfactants, and organic compounds with diverse charge states influenced by pH, while leather effluents often have high salinity and metal complexes. Nanoparticles like CaO can interact differently with these pollutant classes: organic dye molecules may adsorb more readily at neutral pH, while heavy metal complexes may require specific conditions for best removal. Though specific mechanisms vary, the consistent peak at pH 7 in both indicates that neutral pH is broadly favorable for nanoparticle remedial actions in industrial wastewater contexts (Nasir, 2024).

The data in Figure 8 indicated that temperature significantly influences the efficacy of CaO nanoparticle (CaONP) treatment for both textile and leather wastewaters. Both untreated (control) and treated samples exhibited increasing responses up to 30 °C, after which there was a decline as temperature increased to 50 °C. This trend suggested that moderate temperatures enhance pollutant interaction with CaONPs, likely due to increased molecular motion and improved adsorption kinetics, whereas higher temperatures may destabilize nanoparticle–pollutant interactions or promote desorption (Zhou *et al.,* 2024). In particular, CaONP treatment exhibited markedly higher responses than controls at all temperatures, indicating that nanoparticles significantly improve contaminant removal or transformation compared with untreated wastewater. The peak at 30 °C for treated samples (textile: 87 %, leather: 82 %) aligned with findings from similar nanomaterial treatment studies where optimal performance was achieved at moderate temperatures due to favorable reaction kinetics and surface interaction dynamics (Li & Wang, 2025). Above this optimal temperature, reduced performance may be attributed to changes in the physicochemical stability of the nanoparticles or thermal effects on pollutant structure (Smith *et al*., 2024). Moreover, while both wastewater types responded similarly to temperature changes, textile wastewater consistently showed slightly higher values than leather wastewater across all conditions. This may reflect differences in pollutant composition and their interaction mechanisms with CaONPs; textile effluents often contain organic dyes and chemicals that are more amenable to nanoparticle adsorption at elevated temperatures compared with components typically found in leather effluents (Kumar & Patel, 2025).

The data in Figure 9 demonstrated that CaO nanoparticle (CaONP) treatment substantially enhanced the response values of both textile and leather wastewater compared with their respective controls across all tested levels. Control samples exhibited modest increases at lower concentrations but plateaued or declined at higher levels, suggesting limited intrinsic remediation capacity without nanoparticle intervention. In contrast, CaONP-treated samples reached significantly higher responses, with maxima observed at intermediate levels (e.g., textile: 91 % at 1 g/100 mL, leather:

86 % at 2 g/100 mL), indicating that moderate dosage or pollutant concentrations foster optimal nanoparticle performance. These findings aligned with previous research indicating that nanoparticle-mediated remediation often exhibits nonlinear efficiency with respect to dosage or concentration; optimal interaction between nanoparticles and pollutants typically occurs within a specific range, beyond which adsorption sites may become saturated or aggregation of nanoparticles reduces effectiveness (Patel & Singh, 2025). Additionally, textile wastewater generally exhibited higher treatment responses than leather wastewater at similar levels, which could reflect differences in pollutant composition and affinity for CaONP surfaces, as organic dyes and chemicals common in textile effluents may be more readily adsorbed than complex components typical of leather effluents (Zhang & Liu, 2024). The decline in treatment response at the highest level (3 g/100 mL) suggests that excessively high pollutant loads or treatment doses may reduce overall effectiveness, possibly due to nanoparticle aggregation or competition for active sites (Rao *et al*., 2024).

Conclusion

The study demonstrated significant interspecific variability in heavy metal tolerance among the five endophytic strains. Tolerance is metal-specific, concentration-dependent, and likely mediated by diverse physiological and biochemical mechanisms. Strains 1 and 4 appear particularly promising for biotechnological applications in heavy metal remediation due to their high and broad tolerance profiles. The diversity of fungal genera identified suggests a complex endophytic community within the host Moringa plant. Both effluent types showed parameters outside recommended limits for pH, DO, and turbidity, suggesting the need for improved on-site treatment before discharge into the environment. CaO nanoparticles (CaONPs) effectively enhanced the treatment of both textile and leather wastewater. Maximum removal efficiency was observed at neutral pH (7), moderate temperature (30 °C), intermediate concentration/dosage, and 60 minutes' contact time. Treatment performance declined at extreme pH, high or low concentrations, prolonged exposure, and elevated temperatures. Textile wastewater showed slightly higher responses than leather wastewater, likely due to differences in pollutant composition. Overall, CaONP treatment is a promising, efficient, and tunable method for improving industrial wastewater quality.

Conflict of Interest

The authors declare no conflict of interest.

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