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Cavern Whisperers Unveiled: Yemen's Inaugural Speleomycobiome Baseline Across Arid Extremes and Zonation Gradients in Zahzah Cave, Amran

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Abstract:

Cave ecosystems constitute paradigmatic oligotrophic refugia where fungal mycobiomes manifest pronounced zonation along light-nutrient gradients. This inaugural systematic inventory documents cultivable microfungi from Zahzah Cave, Yemen—a hyper-arid karst system absent from speleomycological records. Across 30 rigorously sampled matrices (soil n=12, rock n=10, air n=8) spanning entrance-twilight-dark transects, 78 morphotypes (33 genera) emerged, overwhelmingly dominated by *Aspergillus* (20 spp., 42%) and *Penicillium* (14 spp., 22%) consortia (>64% relative abundance across substrates). Microclimatic forcings—inward cooling (16.1→9.5°C), escalating lithic moisture (0→20.3%), and ventilation attenuation—drove substrate-specific guilds and diversity attrition: twilight soil apices (395×10^3 CFU g^{-1}, S=46, H′=3.45, λ=0.11) versus dark minima (127×10^3 CFU g^{-1}, S=31, H′=2.76); airborne *Cladosporium* hegemony (27-29%, 246→99 CFU m^{-3}); edaphic *Trichoderma*, lithobiontic *Trichophyton*/*Isaria* partitioning. Yemen's seminal integrated cave mycology baseline unveils hyper-arid dynamics—Eurotiales supremacy—divergent from temperate/mesic archetypes, catalyzing phylogenomic and extremozyme interrogation of substrate specialists.

INTRODUCTION

Cave ecosystems embody extreme oligotrophic environments defined by perpetual darkness, consistently low temperatures (typically 10–20°C), high humidity, and severe nutrient scarcity, which impose selective pressures driving microbial evolution toward metabolic efficiency and resilience (Alves *et al.,* 2022; Kosznik-Kwaśnicka *et al.,* 2022; Luis-Vargas *et al.,* 2024; Barbosa *et al.,* 2025). These conditions marked by minimal allochthonous inputs like sporadic guano deposits, drift organic matter or rare organic debris create a stable yet unforgiving habitat where energy capture depends on exploiting fleeting resources, favoring slow-growing, highly adaptable microorganisms capable of long-term dormancy and rapid opportunistic colonization, favoring fungi over bacteria (Simões *et al.,* 2015; Tebo *et al.,* 2015; De Paula *et al.,* 2019; Barbosa, *et al.,* 2025).

Fungi dominate these subterranean niches due to their hyphal growth morphology, which enables extensive exploration of insoluble substrates across vast surfaces, efficiently accessing dispersed carbon and nutrients in air, soil, and rock biofilms (Jiang *et al.,* 2017). Hyphal networks penetrate microfractures and form symbiotic or competitive interactions within cave food webs, often comprising up to 80% of microbial biomass in aphotic zones where bacterial competition is limited by diffusion constraints (Bay *et al.,* 2025). This prevalence reflects evolutionary adaptations to isolation, with spore dispersal via air currents sustaining populations across entrance, twilight, and dark zones (Kosznik-Kwaśnicka *et al.,* 2022).

Distinct zonation patterns emerge along light-nutrient gradients: entrance zones host allochthonous species from surface influx, twilight areas support transitional colonists, and dark interiors favor obligate cavernicoles with enhanced stress tolerance (Barbosa *et al.,* 2025) (Figure 1). Fungi orchestrate nutrient cycling by breaking down recalcitrant organic inputs, supporting detritivores like arthropods and influencing cave geochemistry through mineral interactions, thus maintaining ecosystem stability in Yemen's karstic caves (De Paula *et al.,* 2019; Luis-Vargas *et al.,* 2024).

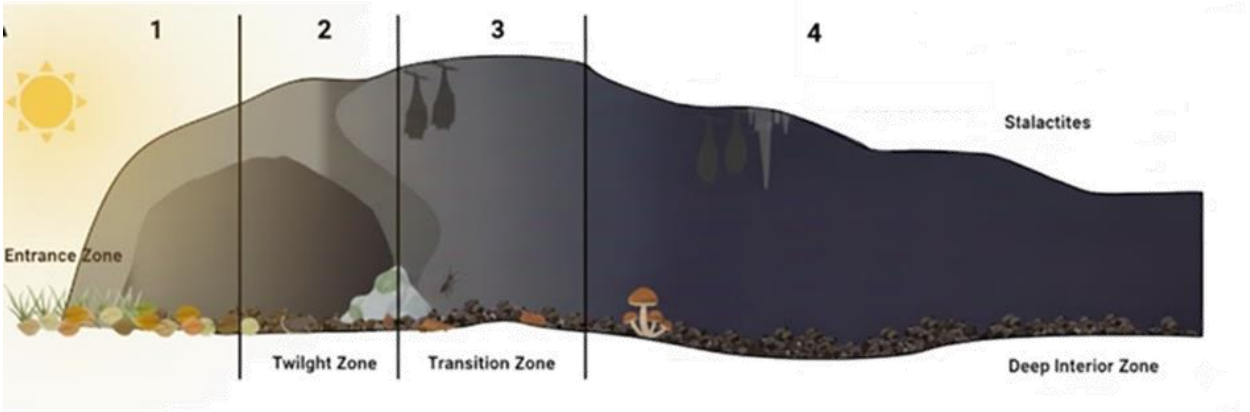

Fig. 1. Schematic representation of cave zones. 1.entrance zone, 2. twilight zone, 3. transition zone, 4.dark zone. Source: Kosznik-Kwa´snicka *et al.,* 2022 (modified) Created with BioRender.com.

This groundbreaking investigation launches Yemen's inaugural speleomycological odyssey into uncharted karst territory—where cultivable cave fungi remain terra incognita amid zero published records. Zahzah Cave emerged as the Yemeni's first quantitative fungal baseline, systematically stratifying soil, rock, and air matrices across canonical entrance-twilight-dark

gradients to resolve substrate-specific guilds and zonation signatures absent from global databases. Thriving within hyper-arid crucibles of episodic dust deposition, pronounced surface-subsurface thermal disequilibria, and negligible precipitation, this dust-fueled system manifests mycobiome dynamics fundamentally divergent from temperate and tropical cave paradigms. As Yemeni's foundational reference, Zahzah Cave established the critical platform for multilocus phylogenetics, extremozyme bioprospecting, and biogeographic modeling of arid-adapted subterranean consortia.

MATERIALS AND METHODS

Study area and sampling design

Sampling transpired within Zahzah Cave (Al-Jaief region, Amran Governorate, Yemen) during May 2024, a hyper-arid system-oriented northwest. Stratified along the principal axis into three canonical zones: entrance (phototrophic, direct allochthonous influx), twilight (penumbral transition), and dark (aphotic profundum) (Figure 2), augmented by proximal outside-air (entrance) and distal inside-air (twilight-dark) benchmarks.

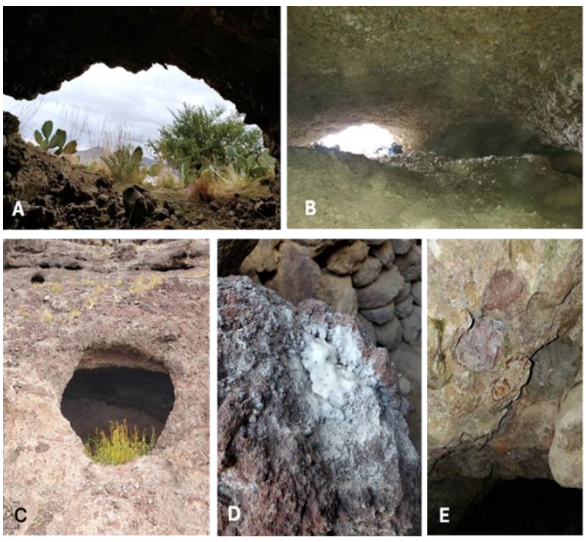

Fig. 2. The Zahzah cave. A. the cave entrance; B. twilight zone; C. a small side opening; D. the cave rocks and E. the cave ceiling in the dark zone.

Thirty rigorously stratified samples included: soil (n=12; 4 per zone), lithic biofilms (n=10; swabbed across entrance-twilight-dark transects), and air (n=8; 4× outside, 4× inside). Microclimatic gradients were quantified via LB-522 thermo-hygrometer (air T/RH), Testo 606-1 (lithic moisture), and Testo 410-1 anemometer (ventilation velocity)—capturing inward cooling, moisture escalation and airflow attenuation driving fungal zonation.

Fungal isolation and enumeration

Soil (10 g) samples were placed into 90 mL of sterile physiological saline (0.85% NaCl) and shaken for 30 min at room temperature before serial ten-fold dilutions (10^{-1}–10^{-4}) were prepared. From each dilution, 0.1 mL was spread, in duplicate, onto Potato Dextrose Agar (PDA; 200 g potato infusion, 20 g dextrose, 15 g agar per liter, pH 5.6) supplemented with chloramphenicol (50 mg/L) to suppress bacteria. Rock-associated fungi were sampled by swabbing a defined wall area from all zones (e.g. 10 × 10 cm) with sterile cotton swabs pre-moistened in physiological saline; each swab was then straked on PDA plates. Airborne fungi were sampled using the settle-plate method by exposing PDA plates at ~1.0 m above the floor for 30 min at each outside-air and inside-air station, with three replicate plates per station. All plates were incubated at 25 ± 3 °C in the dark for 5–7 days and examined daily for colony development; incubation was extended up to 10 days for slow-growing isolates; Colony-forming units (CFU) were counted on plates with 30–300 colonies and expressed as CFU/g (soil), CFU/cm² equivalent (rock swabs), or CFU/m³ (air).

Fungal identification

Isolates were purified by repeated subculture on PDA until single-morphotype colonies were obtained, then identified primarily using macro- and micro-morphological criteria. Colony colour, texture, exudate production, and radial growth were recorded after 7 days, and microscopic structures (conidiophores, phialides, conidia, sporangia) were examined from

lactophenol cotton blue mounts using a compound microscope. Identification was assigned using appropriate taxonomic guides and recent literature (Raper and Fennell 1965; Raper and Thom 1968; Zycha and Siepmann 1969; Ellis, 1971; 1976; Arx, 1974; Gilman, 1975; Raper and Fennel, 1977; Pitt, 1979; 1991; Domsch *et al.,* 1980; Ramirez, 1982; Sivanesan, 1984; Seifert and Samson, 1986; Moubasher, 1993; Echevarría and Iqbal, 2021). When isolates could not be unambiguously assigned to a species based on morphology, they were retained at genus level and reported as, for example, *Fusarium* sp., *Mucor* sp., or *Chrysosporium* sp.

RESULTS

Microclimate

Zahzah Cave showed expected single-entrance gradients: air temperature progressively declined from entrance to dark (16.1 to 9.5°C), wind speed weakened inward (1.2 to 0.4), and rock moisture increased (0 to 20.3%), despite low RH (38-45%) typical of arid karst systems (Table 1).

Table 1. Microclimate conditions in the cave.

Location of measurement	Air			Rocks
	Temperature C	Relative humidity %	Wind speed [m/s]	Moisture content %
Entrance zone	16.1	38%	1.2	0.0
Twilight Zone	13.2	41%	0.7	19.2
Dark Zone	9.5	45%	0.4	20.3

Community Composition in the Cave

Zahzah Cave mycobiomes manifested pronounced substrate- and zonation-specific structuring across 78 morphotypes (33 genera): soil (62 spp., 24 genera), air (47 spp., 16 genera), lithic biofilms (33 spp., 15 genera) spanning entrance (phototrophic influx), twilight (penumbral transition), dark (aphotic profundum), and proximal/distal air benchmarks (Table, 2). Eurotiales hegemony prevailed universally, *Aspergillus* (20 spp., 42%), *Penicillium* (14 spp., 22%), >64% relative abundance, with *A. niger* and *P. chrysogenum* omnipresent across gradients.

Airborne consortia were *A. longipes* and Cladosporium-enriched (18% outside, 22% inside); edaphic exclusives (*A. alliaceus, A. iizukae, Chaetomium sp., Curvularia sp., P. vulpinum, T. harzianum, T. viride, R. stolonifer*) signified organic-specialist guilds; lithobiontic specialists (*Isaria fumosorosea, Trichophyton sp., yeasts*) partitioned to rock matrices. Interiorward habitat specialization intensified, crystallizing substrate guilds along light-nutrient attenuation vectors.

Table 2. Microfungi isolated from the entrance, twilight and dark zones soil, outside and inside air and the rocks surfaces in the cave.

Fungal species	Soil			Air		Rocks (walls)
	Entrance zone	Twilight zone	Dark zone	Outside	Inside	
Absidia sp.	+*	+	-	-	-	-
Acremonium sp.	-*	+	-	-	-	-
Alternaria alternata	+	+	-	+	+	-
A. longipes	-	-	-	+	-	-
A. tenuissima	+	+	-	+	-	+
Aspergillus alliaceus	+	-	+	-	-	-

A. bertholletiae	-	+	-	-	+	+
A. candidas	+	-	+	+	-	-
A. creber	-	-	-	+	-	+
A. flavus	+	+	+	-	+	-
A. fumigatus	+	+	+	+	-	-
A. iizukae	+	-	+	-	-	-
A. japanicus	+	-	+	-	-	+
A. jensenii	+	-	-	+	-	-
A. niger	+	+	+	+	+	+
A. ochraceus	-	+	+	+	-	+
A. parastiticus	+	+	-	-	-	+
A. sesamicola	-	-	-	+	+	-
A. sydowii	+	-	+	+	-	-
A. tamarii	-	+	+	+	-	+
A. terreus	+	+	+	-	-	+
A. usts	+	-	-	+	+	+
A. versicolor	+	-	-	+	-	+
A. wentii	-	+	-	+	-	+
Aspergillus sp.	-	+	-		-	-
Aureobasidium pullulans	+	+	+	+	-	-
Botrytis cinerea	-	-	-	+	+	-
Chaetomium sp.	+	+	+	-	-	-
Chrysosporium sp.	-	+	-	-	-	+
Cladosporium bruhnei	-	-	-	+	-	-
Cl. cladosporioides	-	-	-	+	+	-
Cl. herbarum	-	-	-	+	+	-
Clonostachys candelabra	-	+	-	+	-	+
Cunninghamella echinulata	+	-	-	-	-	-
C. elegans	-	+	+	-	-	-
Curvularia sp.	-	+	-	-	-	-
Embellisia abundans	+	+	-	-	-	-
Epicoccum nigrum	-	-	-	+	+	-
Fusarium graminearum	+	-	+	-	+	+
F. oxysporum	-	-	+	+	+	-
F. solani	+	-	+	-	+	+
Fusarium sp.	+	+	+	-	+	+
Geoterichum candidum	-	+	-	-	-	+
Humicola seminuda	-	-	+	-	-	-
Isaria fumosorosea	-	-	-	-	-	+
Lecanicillium psalliotae	+	+	-	-	-	+
Mortierella sp.	-	+	+	-	-	+
Mucor circinelloides	+	+	-	+	-	+
M. hiemalis	+	+	+	+	+	+
M. luteus	+	+	-	-	-	-
Myceliophthora sp.	-	+	-	-	-	-
Paecilomyces fumosoroseus	-	+	-	+	-	-
Penicillium brevicompactum	-	+	+	+	-	-
P. chrysogenum	+	+	+	+	+	+
P. citrinum	+	-	+	+	-	-
P. commune	+	+	-	+	-	-
P. cyclopium	+	+	+	+	-	+
P. expansum	-	-	-	+	+	-
P. glabrum	-	+	-	-	+	-
P. purpurogenum	-	+	-	-	-	+
P. spinulosum	-	-	+	+	+	+
*P. swiecick*ii	-	-	-	+	+	-
P. variabile	-	+	-	+	-	+
P. viridicatum	-	+	-	+	-	+
P. vulpinum	+	+	+	-	-	-
Penicillium sp.	+	-	-	-	-	-
Phoma herbarum	+	+	-	-	-	-
Rhizopus arrhizus	-	+	-	+	-	-
R. stolonifer	+	+	+	-	-	-

Sarocladium terricola	-	-	-	+	+	-
Simplicillium lamellicola	-	-	-	-	-	+
Stachybotrys chartarum	+	+	+	+	-	+
Talaromyces allahabadensis	-	-	-	+	-	-
Trichoderma harzianum	+	+	+	-	-	-
T. koningii	+	+	-	+	-	-
T. viride	-	+	+	-	-	-
Trichophyton sp.	-	-	-	-	-	+
Yeast	-	-	-	-	-	+
Σ genera/zone		24		16		15
Σ species/zone		62		47		33
Σ genera	33					
Σ species	78					

*"+" species present; "-" species absent.

Substrate-Specific Diversity Patterns

Soil Microfungi Diversity

Soil fungal loads peaked dramatically in the twilight zone (395 × 10^3 CFU g^{-1}, S=46), surpassing entrance (237 × 10^3 CFU g^{-1}, S=38) and dark zone (127 × 10^3 CFU g^{-1}, S=31) minima—quantifying >3-fold zonation gradient (H′=3.45 to 2.76). *Aspergillus* hegemony intensified interiorward: *A. flavus* (10% entrance, 8% twilight) yielded to oligotrophic *A. niger* (11% dark), emblematic of progressive specialist partitioning along nutrient attenuation vectors (Table 3).

Table 3. Total count and percentage of microfungi isolated from the entrance, twilight and dark zones soil of the cave (CFU/g).

Fungal species	Sampling location (TC (CFU/g) ×10^3)					
	Entrance Zone		Twilight zone		Dark zone	
	TC	TC %	TC	TC %	TC	TC %
Abasidia sp.	10	4	2	1	-	-
Acremonium sp.	-	-	3	1	-	-
Alternaria alternata	2	1	2	1	-	-
A. tenuissima	2	1	6	2	-	-
Aspergillus alliaceus	2	1	-	-	3	2
A. bertholletiae	-	-	2	1	-	-
A. candidas	1	0	-	-	2	2
A. flavus	23	10	30	8	2	2
A. fumigatus	17	7	26	7	10	8
A. iizukae	3	1	-	-	6	5
A. japanicus	3	1	-	-	5	4
A. jensenii	4	2	25	6	-	-
A. niger	11	5	4	1	14	11
A. ochraceous	-	-	4	1	2	2
A. parastiticus	15	6	8	2	-	-
A. sydowii	7	3	-	-	5	4
A. tamarii	-	-	9	2	3	2
A. terreus	3	1	2	1	4	3
A. usts	5	2	-	-	-	-
A. versicolor	3	1	-	-	-	-
A. wentii	-	-	3	1	-	-
Aspergillus sp.	1	0	-	-	-	-
Aureobasidium pullulans	2	1	28	7	1	1
Chaetomium sp.	8	3	4	1	5	4
Chrysosporium sp.	-	-	10	3	-	-

Clonostachys candelabra	-	-	3	1	-	-
Cunninghamella echinulate	4	2	-	-	-	-
C. elegans	-	-	15	4	3	2
Curvularia sp.	-	-	15	4	-	-
Embellisia abundans	2	1	2	1	-	-
Fusarium graminearum	3	1	-	-	3	2
F. oxysporum	-	-	-	-	3	2
F. solani	3	1	-	-	2	2
Fusarium sp.	11	5	19	5	5	4
Geoterichum candidum	-	-	2	1	-	-
Humicola seminuda	-	-	-	-	3	2
Lecanicillium psalliotae	3	1	1	0	-	-
Mortierella sp.	-	-	10	3	3	2
Mucor circinelloides	6	3	2	1	-	-
M. hiemalis	9	4	12	3	5	4
M. luteus	8	3	9	2	-	-
Myceliophthora sp.	-	-	3	1	-	-
Paecilomyces fumosoroseus	-	-	14	4	-	-
Penicillium brevicompactum	-	-	3	1	2	2
P. chrysogenum	6	3	14	4	1	1
P. citrinum	3	1	-	-	6	5
P. commune	9	4	5	1	-	-
P. cyclopium	-	-	12	3	3	2
P. glabrum	-	-	3	1	-	-
P. purpurogenum	-	-	12	3	-	-
P. spinulosum	-	-	-	-	3	2
P. variabile	-	-	5	1	-	-
P. viridicatum	-	-	6	2	-	-
P. vulpinum	11	5	12	3	9	7
Penicillium sp.	1	0	-	-	-	-
Phoma herbarum	3	1	1	0	-	-
Rhizopus arrhizus	-	-	3	1	-	-
R. stolonifer	8	3	12	3	1	1
Stachybotrys chartarum	10	4	12	3	9	7
Trichoderma harzianum	12	5	15	4	3	2
T. koningii	3	1	2	1	-	-
T. viride	-	-	3	1	1	1
Σ Isolates/zone	237		395		127	

Air and rocks microfungi diversity

Airborne fungal loads plummeted 60% interiorward (246 CFU m^{-3}/41 spp. outside to 99 CFU m^{-3}/21 spp. inside), overwhelmingly dominated by *Cladosporium* spp. (9% outside, 19% inside), quantifying rigorous ventilation filtering of allochthonous spore influx. Lithic biofilms averaged 127 CFU cm^{-2} (33 spp.), sustaining *Penicillium-Aspergillus* hegemony augmented by oligotrophic specialists (*Isaria fumosorosea*, *Trichophyton* sp., yeasts), emblematic of moisture-mediated rock colonization (Table 4).

Table 4. Total count and percentage of microfungi isolated from outside and inside air and rock surface samples of the cave (CFU/m^3) and (CFU/cm^2), respectively.

Fungal species	TC (CFU/m^3)				TC (CFU/cm^2)	
	Air				Rock surface (walls)	
	Outside		Inside			
	TC	TC %	TC	TC %	TC	TC %
Alternaria alternata	21	9	3	3	-	-
A. longipes	2	1	-	-	-	-

A. tenuissima	8	3	-	-	5	4
A. bertholletiae	-	-	3	3	1	1
A. candidas	5	2	-	-	-	-
A. creber	3	1	-	-	2	2
A. flavus	-	-	3	3	-	-
A. fumigatus	12	5	-	-	-	-
A. japanicus	-	-	-	-	4	3
A. jensenii	2	1	-	-	-	-
A. niger	10	4	3	3	8	6
A. ochraceous	3	1	-	-	5	4
A. parastiticus	-	-	-	-	1	1
A. sesamicola	2	1	1	1	-	-
A. sydowii	2	1	-	-	-	-
A. tamarii	1	0	-	-	1	1
A. terreus	-	-	-	-	2	2
A. usts	10	4	6	6	4	3
A. versicolor	4	2	-	-	3	2
A. wentii	2	1	-	-	2	2
Aureobasidium pullulans	3	1	-	-	-	-
Botrytis cinerea	2	1	1	1	-	-
Chrysosporium sp.	-	-	-	-	9	7
Cladosporium bruhnei	5	2	-	-	-	-
Cl. Cladosporioides	22	9	19	19	-	-
Cl. herbarum	16	7	10	10	-	-
Clonostachys candelabra	7	3	-	-	3	2
Epicoccum nigrum	6	2	4	4	-	-
Fusarium graminearum	-	-	2	2	6	5
F. oxysporum	3	1	2	2	-	-
F. solani	-	-	4	4	5	4
Fusarium sp.	-	-	10	10	2	2
Geoterichum candidum	-	-	-	-	2	2
Isaria fumosorosea	-	-	-	-	2	2
Lecanicillium psalliotae	-	-	-	-	2	2
Mortierella sp.	-	-	-	-	3	2
Mucor circinelloides	3	1	-	-	1	1
M. hiemalis	6	2	2	2	3	2
Paecilomyces fumosoroseus	4	2	-	-	-	-
Penicillium brevicompactum	5	2	-	-	-	-
P. chrysogenum	13	5	10	10	22	17
P. citrinum	5	2	-	-	-	-
P. commune	4	2	-	-	-	-
P. cyclopium	3	1	-	-	1	1
P. expansum	4	2	3	3	-	-
P. glabrum	-	-	2	2	-	-
P. purpurogenum	-	-	-	-	7	6
P. spinulosum	10	4	3	3	1	1
P. swiecickii	6	2	4	4	-	-
P. variabile	9	4	-	-	5	4
P. viridicatum	2	1	-	-	1	1
Rhizopus arrhizus	5	2	-	-	-	-
Sarocladium terricola	6	2	4	4	-	-
Simplicillium lamellicola	-	-	-	-	1	1
Stachybotrys chartarum	6	2	-	-	8	6
Talaromyces allahabadensis	2	1	-	-	-	-
Trichoderma koningii	2	1	-	-	-	-
Trichophyton sp.	-	-	-	-	3	2
Yeast	-	-	-	-	2	2
Σ Isolates/zone	246		99		127	

Richness, Diversity & Dominance

Zahzah Cave mycobiomes exhibited pronounced diversity gradients across substrates, peaking in twilight-zone soil (S=46, H′=3.45, 365 isolates) and outside air (S=41), intermediate in entrance/dark soils and lithic biofilms (S=31–38), and nadir in inside air (S=21, H′=2.18, 99 isolates)—crystallizing classic single-entrance zonation (Table 5). Twilight soil epitomized diversity apices: maximal richness, equitability (λ=0.11), and abundance, signifying transitional nutrient/light convergence fostering expansive, evenly distributed assemblages. Conversely, inside air manifested dominance consolidation (λ=0.37) among sparse, resilient airborne taxa, emblematic of rigorous environmental filtering. Rock surfaces and dark-zone soils sustained moderate richness/diversity despite isolate paucity, reflecting oligotrophic specialist guilds with compositional evenness adapted to profundal cave rigors (Figures 3 and 4).

Table 5. Microfungi species richness, diversity and dominance in each cave site.

Location	Total isolates ↓	Richness ↓	Shannon Index (H′, ln) ↑	Dominance (λ) ↓ Diversity ↑	Notes
Soil (twilight)	365	46	3.45	0.11	diversity peak
Air (outside)	246	41	3.28	0.14	surface influx
Soil (entrance)	237	38	3.12	0.16	allochthonous input
Rocks	127	33	2.89	0.21	specialized
Soil (dark)	127	31	2.76	0.24	oligotrophs
Air (inside)	99	21	2.18	0.37	filtered

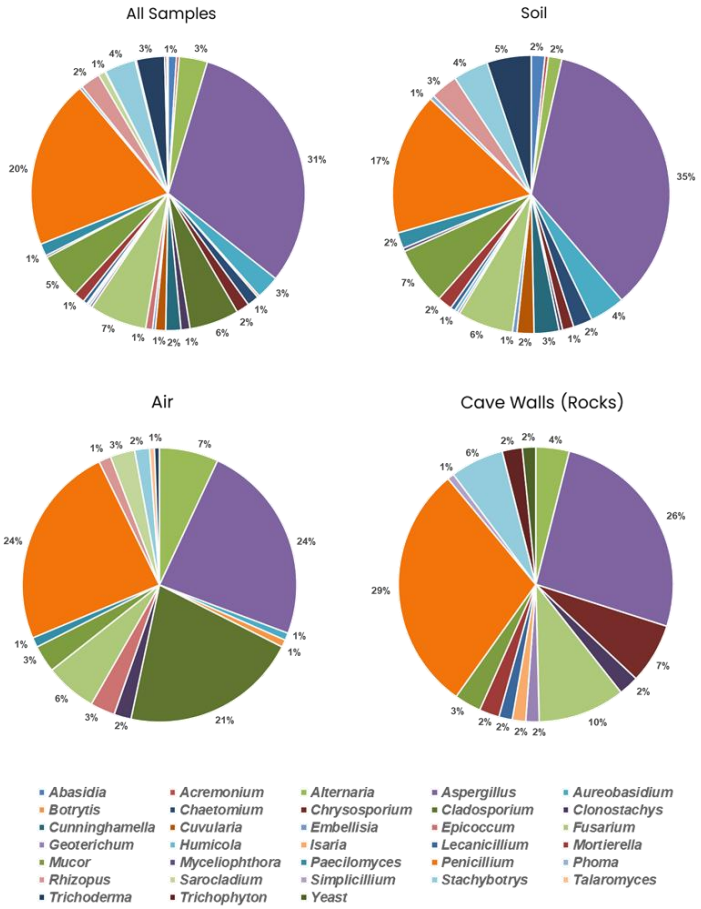

Fig. 3. Relative abundance of microfungal genera in Zahzah Cave. a. all samples; b. soil samples; c. air samples and d. cave walls (rock surface) samples.

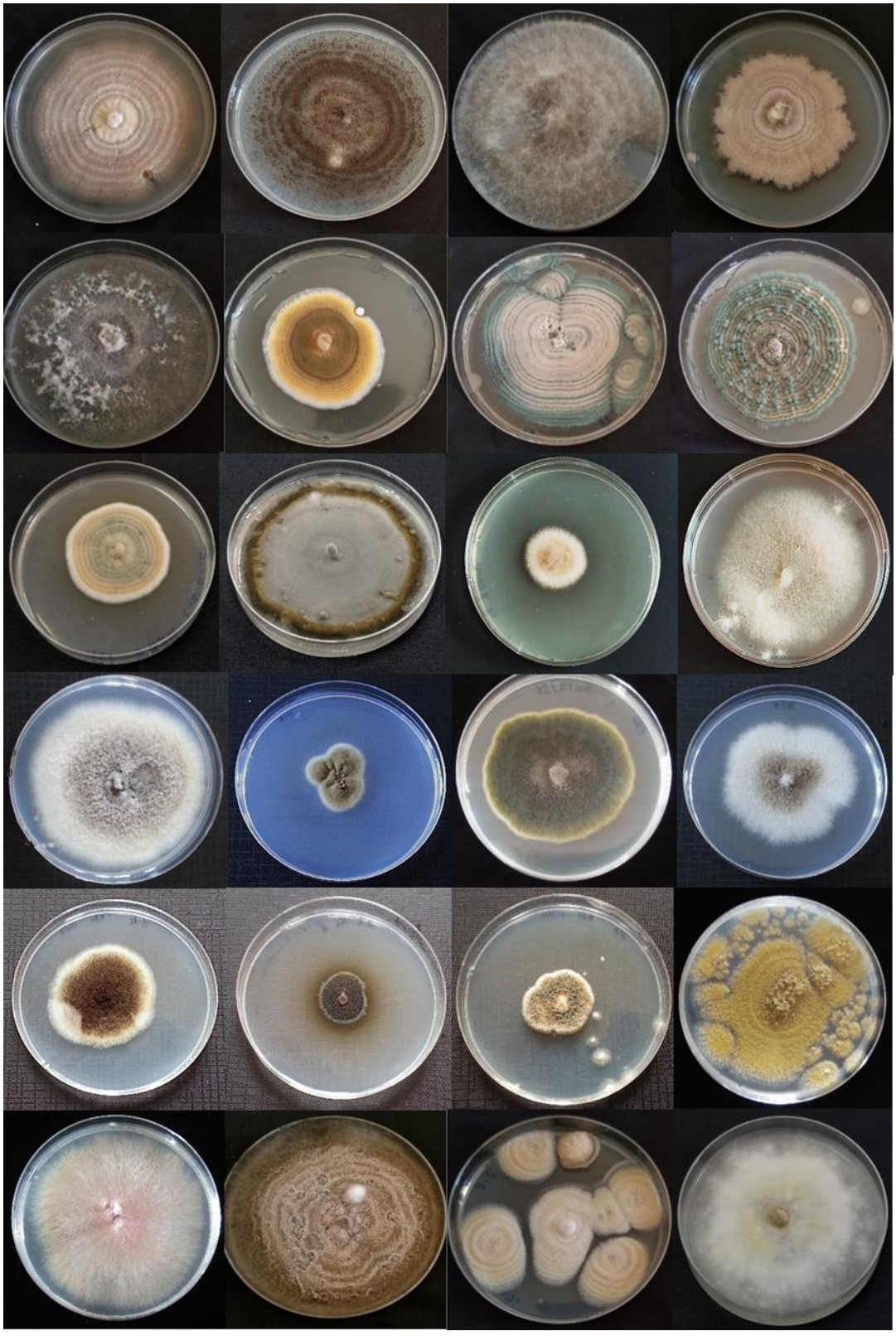

Fig. 4. The diversity of genera isolated from Zahzah Cave in different substrates.

DISCUSSION

Zahzah Cave's microclimatic gradients,inward cooling (16.1 to 9.5°C), escalating relative humidity, ventilation attenuation, and lithic moisture escalation (0 to 20.3%), precisely recapitulate canonical single-entrance cave dynamics documented globally. Entrance air temperatures track external fluctuations with diminished amplitude, stabilizing interiorward toward mean annual surface isotherms, mirroring temperate/tropical paradigms (Medina *et al.,* 2023). Relative humidity surges to near-saturation in profundal zones via wall/sediment evaporation, contrasting entrance desiccation and airflow exchange; air velocity plummets, fostering stasis (Zelinka, 2002; Pflitsch and Piasecki, 2003; D'Agostino *et al.,* 2015). Dry photic lithics yield to condensation-corroded, capillary-sustained inner walls, nucleating microbial/fungal biofilms under cool, saturated equilibria (Piano *et al.,* 2015). Overall, Zahzah's data align with classic cave zonation models, promoting distinct communities in the humid, persistent-moisture interior versus the entrance zone.

Community Composition in the Cave

Zahzah Cave's fungal mycobiome manifested unequivocal zonation along microclimatic and substrate gradients, recapitulating global cave paradigms while inaugurating Yemen's inaugural comprehensive inventory; 78 morphotypes (33 genera): soil (62 spp.), air (47 spp.), lithics (33 spp.), filling a critical void in global arid speleomycology (Jiang *et al.,* 2017; Nováková *et al.,* 2018; Alves *et al.,* 2022; Lima *et al.,* 2024; Poli *et al.,* 2024; dos Prazeres *et al.,* 2025). Edaphic matrices reservoir maximal richness as primary diversity sinks; aerial/lithic interfaces mediate dispersal/colonization. Ascomycota anamorphs, Eurotiales hegemony (*Aspergillus* 20 spp., *Penicillium* 14 spp.; >64%), prevailed alongside *Fusarium*, *Alternaria*, *Cladosporium*, *Mucor*, *Trichoderma*, mirroring Italian/Brazilian/Spanish cave meta-patterns (Alves *et al.,* 2022; Cunha *et al.,* 2020; Dominguez-Moñino *et al.,* 2021; Poli *et al.,* 2024). *A. niger* and *P. chrysogenum* exhibited pan-zonal, cross-substrate ubiquity, epitomizing oligotolerant generalism: exploiting eutrophic entrance alluvia to profundal nutrient refugia consistent with their stress-resilient profiles in tropical/temperate aerobiology, edaphons, and guano consortia (Lima *et al.,* 2024; Burazerović *et al.,* 2025).

Substrate-Specific Diversity Patterns

Soil Microfungi Diversity

Aspergillus flavus and *A. fumigatus* dominated entrance/twilight soils, while *A. niger* prevailed in the dark zone, establishing a resilient core *Aspergillus* assemblage spanning light-nutrient gradients (Hsu and Agoramoorthy, 2001; Visagie *et al.,* 2021). Twilight peaks in isolate density and species richness plummeted interiorward (>70% decline) under stringent environmental filtering and dwindling organic inputs, selectively enriching oligotrophic specialists like *A. niger*, *P. vulpinum*, and *S. chartarum* (Cunha *et al.,* 2020; Poli *et al.,* 2024). Persistent *Aspergillus–Penicillium* hegemony across all zones—bolstered by twilight satellites (*P. fumosoroseus*) and dark-zone endemics (*A. iizukae, P. citrinum*)—exemplifies classic generalist-to-specialist zonation, paralleling Eurotiales-dominated cave mycobiomes worldwide (Cunha *et al.,* 2020; Visagie *et al.,* 2021). Similarly, soil-exclusive taxa (*A. alliaceus, A. iizukae, Chaetomium* sp., *Curvularia* sp., *P. vulpinum, Trichoderma* spp., *R. stolonifer*) formed distinct organic-specialist guilds, thriving in enzyme-rich microhabitats fueled by particulates, debris, and guano (Cunha *et al.,* 2020).

Air Microfungi Diversity

C. cladosporioides dominated airborne assemblages at entrance and interior zones, underscoring its prolific spore production and profound adaptation to cave aerobiology. *A. alternata* (9% outside air) traced primarily to exogenous surface vegetation, exhibiting negligible persistence interiorward (Docampo *et al.*, 2011). Endogenous taxa, *C. herbarum*, *Fusarium* sp. and *P. chrysogenum* (~10% each) persisted through chronic resuspension from soil

reservoirs, guano accumulations, and biofilm matrices, recapitulating the archetypal *Penicillium-Cladosporium* hegemony observed in Nerja and Heshang caves (Ogórek *et al.,* 2014; Zhang *et al.,* 2015). Aerial propagule guild including *A. longipes*, *B. cinerea*, *C. cladosporioides*, *C. herbarum*, *P. expansum*, *P. swiecickii*, *S. terricola* and *T. allahabadensis* manifested exclusively as allochthonous incursions, devoid of lithic/sedimentary colonization and emblematic of cosmopolitan show cave and wild karst mycobiomes (Pflitsch and Piasecki, 2003; Medina *et al.,* 2023). Airborne density/richness plunged 60% (246 CFU/m³/41 spp. to 99 CFU/m³/21 spp.) interiorward, exemplifying rigorous ventilation-mediated filtering of surface-derived spores (interior), illustrating ventilation filtering of surface spores (Pflitsch and Piasecki, 2003; De Freitas and Schmekal, 2003; Docampo *et al.,* 2011).

Rocks Microfungi Diversity

Zahzah Cave walls sustained a stratified mycobiota, overwhelmingly dominated by *P. chrysogenum* (17% CFU/cm²), emblematic of its prodigious sporulation, robust mineral adhesion, and resilience to moisture/nutrient disequilibria quintessential of lithobiontic pioneers (Jurado *et al.,* 2010; Visagie *et al.,* 2014; Zhang *et al.,* 2015). Intermediate opportunists (*A. tenuissima, A. japanicus, A. ochraceus, A. ustus, F. graminearum, F. solani, P. variabile*) colonized discrete micro-niches—microcracks, hygroscopic salt efflorescences, and organic particulates—forming patchy lithic biofilms (Ogórek *et al.,* 2014), Rare taxa (*Isaria fumosorosea*, *Trichophyton* sp., yeasts; 1-2%) exploited ephemeral niches via arthropod necromass, keratinic detritus, and condensed water films, mirroring entomopathogenic/keratinophilic guilds documented in bat guano-rich and quartzite karsts globally (Wasti *et al.*, 2021; Lima *et al.,* 2024; Burazerović *et al.,* 2025).

Richness, Diversity & Dominance

Twilight soil and entrance air constituted unequivocal diversity pinnacles—elevated richness, maximal H′ (3.45), minimal λ (0.11)—with equitably distributed assemblages (Adetutu *et al.,* 2011); Interior air exhibited stark H′ attrition (2.18) via dominance consolidation (λ=0.37) among resilient taxa, recapitulating aerobiological paradigms wherein distal cave air filters entrance spore pools to cave-adapted subsets (Docampo *et al.,* 2011; Ogórek *et al.,* 2014); Rock surfaces and dark-zone soils harbored moderate, oligotrophic Eurotiomycetes consortia, aligning with global meta-analyses documenting substrate-mediated filtering in lithic and profundal cave matrices (Jurado *et al.,* 2010; Burazerović *et al.,* 2025). Wall mycobiota imposed stringent *Penicillium-Aspergillus* hegemony amid transient specialists, crystallizing cave-specific zonation orchestrated by light/nutrient/substrate gradients—cosmopolitan generalists yielding to partitioned guilds (Poli *et al.,* 2024).

This pioneering investigation unveils Yemen's subterranean fungal frontier—the inaugural systematic survey of cultivable cave mycobiota within a speleomycologically pristine Arabian landscape devoid of prior records. Zahzah Cave establishes the quantitative gold standard, rigorously delineating 78 morphotypes across soil, rock, and air matrices through exhaustive entrance-twilight-dark transects, unmasking substrate-specific guilds and unprecedented diversity apices (H′=3.45, twilight soil) in hyper-arid karst. Thriving under extreme desiccation—episodic dust incursions, thermal gradients (16.1–9.5°C), and ephemeral inflows—this forge sculpts mycobiomes radically divergent from temperate/tropical archetypes, dominated by Eurotiales (64%) mastery of oligotrophy. South Arabia's seminal speleomycological atlas, Zahzah galvanizes forthcoming multilocus phylogenomics, extremozyme bioprospecting, and biogeographic insights from Yemen's veiled karst repositories.

CONCLUSION

Zahzah Cave sustains a stratified fungal mycobiome (78 morphotypes, 33 genera) manifesting archetypal single-entrance zonation: twilight soil diversity apices (S=46, H′=3.45, λ=0.11), airborne Cladosporium hegemony, edaphic Trichoderma guilds, and lithobiontic Isaria fumosorosea, orchestrated by microclimatic gradients (16.1–9.5°C), episodic dust pulses, and substrate-mediated filtering. This inaugural integrated (soil/rock/air) inventory from Yemeni karst establishes a rigorous quantitative baseline, illuminating hyper-arid mycobiome dynamics—Eurotiales supremacy (64% relative abundance)—fundamentally divergent from temperate/mesic paradigms. Prospective multilocus phylogenomics of substrate specialists, coupled with extremozyme assays of dominant Eurotiales, will interrogate regional endemism and biotechnological valorization within climate-resilient subterranean refugia.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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