Indian J. Applied & Pure Bio. Vol. 38(2), 887-894 (2023). **ISSN: 0970-2091**
A web of Science Journal

Fungal spore content of the atmosphere of the Cave of Bhaja caves, Lonavala, Maharashtra

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Abstract

Intramural environment of caves contains variety of bio components e.g., micro-organisms, fungal mycelia & spores which may cause harm to the internal environment of caves & health of its visitors including most commonly dwelling bats in the caves. Microbial air contaminants emit secondary metabolites such as mycotoxins, endotoxins, enterotoxins & enzymes that may trigger allergy & adversely effects human health. The presence of microorganisms & visits by tourists can both result in changes to the microclimatic conditions of a cave. The visitors can also be source of organic matter, which can promote growth of microbes & fungal mycelia.

The present study focused on quantitative data of fungal spores inside caves & its adverse effects. Bhaja caves were selected as site due to large no. of visitors in the caves. Total 22 fungal species were recorded inside the cave atmosphere with *Rhizopus* contributing the maximum fungal type. *Rhizopus-17.14% Cladosporium-11.36%, Aspergillus fumigatus-5.61%, Chaetomium-5.14% & Aspegillus-nidulans-5.09%* were most recorded fungal species in the caves. Most fungal colonies were reported during the months of June to Aug 2019.

Key words : Caves, Fungi, allergy, bio components.

The study of the behavior of airborne fungi in caves is of interest because many caves contain Paleolithic paintings that should be protected from fungal outbreaks[7]. Fungal spores represent a potential risk to the conservation of cultural heritage since, in favorable cave environmental conditions (high relative humidity, stable temperature, nutrient inputs, *etc.*), fungi colonize speleothems and other mineral substrata in addition to the cadavers of animals and arthropods populating the cavity[1]. Airborne fungi are transported from outside the cave to the inside because a door opening favors communication with the

external atmosphere and the passage of warm air moves towards the interior of the cave. Intramural environment of caves contains variety of bio components *e.g.*, micro-organisms, fungal mycelia & spores which may cause harm to the internal environment of caves & health of its visitors including most commonly dwelling bats in the caves. Microbial air contaminants emit secondary metabolites such as mycotoxins, endotoxins, enterotoxins & enzymes that may trigger allergy & adversely effects human health[31]. The presence of microorganisms & visits by tourists can both result in changes to the microclimatic conditions of a cave. The visitors can also be source of organic matter, which can promote growth of microbes & fungal mycelia. For the preparation of the manuscript relevant literature[1-40] has been consulted.

The study area :

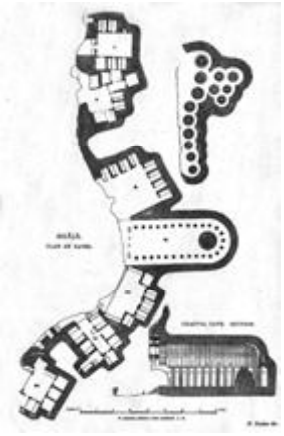
Bhaja Caves is a Caverns & Caves place is in Lonavala, Maharashtra. The latitude of Bhaja Caves is 18.74681, and the longitude is 73.40791. Bhaja Caves is in Lonavala, India with the GPS coordinates of 18° 44' 48.516" N and 73° 24' 28.476" E. The caves, 18 in number, are beautifully sculptured with many images of different postures of Buddha, some animals, and situations of some stories of the tales of Buddhism. Cave 12th is a prayer hall and represents a fantastic example of ancient Buddhist architecture. As usual with cave culture, there are many Viharas in Bhaja also, which, served as monastery for the learning and meditating Buddhist monks. They are one of the oldest among all caves in India. There are stupas, a common feature to all the caves.

Selection of site :

Initially a detailed survey of Bhaja caves and surrounding area was undertaken to select the site, keeping in mind the objectives of the study. This resulted in the selection of site as Bhaja Caves.

Petri plate culture method :

Petri plates containing Rose Bengal Streptomycin (RBS) Agar medium were



exposed once a month for 10 minutes at a height of 2meters from ground level. Three exposures/trappings were done in a day at 8.00hrs,12.00hrs and 16.00hrs, once a month for one year. The RBS Agar medium consisting of the following ingredients was prepared as follows:

Rose Bengal Dye -0.05gm

Bacto-Peptone -2.00gm

Bacto-Agar -20.00gm

Glucose -10.00gm

Magnesium Sulphate -0.50gm

Potassium Dihydrogen Phosphate -0.50gm

Distilled water -1000ml.

All the above ingredients were mixed in a beaker by adding Distilled water and were boiled in a water bath. It was continuously stirred with a glass rod. Later, it was sterilized by autoclaving at a pressure of 15 lbs. for 20 minutes. Soon after cooling the medium to about 45° C in an incubator, streptomycin sulphate 40 units and crystalline penicillin 20 units were added and stirred under sterile environment. The medium was then poured into 10 cm diameter petri dishes, each containing 20 ml medium covered with Petri lid and taped immediately, under aseptic conditions. After cooling and solidifying for about 2 hours, the petri-plates were then stored at room temperature for 3 days. These were then examined for the growth of any contaminants and the selected Petri-plates were then taken to the sites for exposure. After exposure for 10 minutes each Petri-plate was immediately covered with lid and taped. These were then taken to the laboratory and incubated at 28° C to 30° C in an inverted position for 7 days. The fungal colonies developed were identified at the generic level from their characteristic branching of conidiophores, morphology of spores and sporulation. These were compared with the reference slides and standard illustrations. The fungal colonies were also subcultured on PDA slants and were sent for identification to Agharkar Research Institute, Pune.

This method had the advantage over the gravity slide sampling in that while the latter method could not identify the small, rounded spores to their genera due to similarities in their morphology. With the Petri-plate method these spores germinated to develop into colonies. These colonies showed distinct conidiophores or branching characteristic of the various genera of fungi producing small, rounded spores, along with colonies of other genera having spores of distinct morphological identities[2].

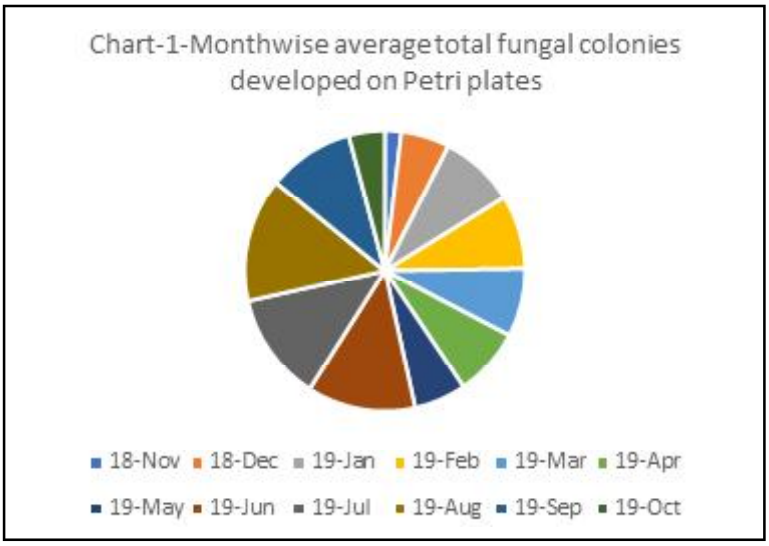
Fungal identification :

The fungal colonies grown on all the Petri dishes were counted and identified. The specific identification of the sampled fungi was performed using macro- and microscopic observations, namely the morphology of hyphae, conidia, and sporangia, of the colonies that had grown on the culture media according to the commonly accepted methods used in mycological laboratories. The fungi were identified using diagnostic keys.

Total 22 species of fungi were recorded during one year of study from Nov 2018 to Oct 2019 in the indoor environment of Bhaja Caves in Lonavala, Maharashtra. Following are the recorded fungal species & their percentage concentration.

Table-1. Petri plate Culture Method
Month wise average total fungal colonies developed on Petri Plates-
(Indoor Environment of caves)

Site	Nov 2018	Dec 2018	Jan 2019	Feb 2019	March 2019	April 2019	May 2019	June 2019	July 2019	Aug 2019	Sep 2019	Oct 2019
Plate18.00 Hrs.	04	09	13	17	14	13	11	22	24	21	15	09
Plate212.00 Hrs.	03	07	13	13	15	8	10	18	17	23	17	05
Plate316.00 Hrs.	02	11	16	11	9	17	8	20	19	25	16	06
Average Colonies developed	**3**	**9**	**14**	**13.66**	**12.66**	**12.66**	**9.6**	**20**	**20**	**23**	**16**	**6.66**



Alternaria alternata :

Conidia are typically obclavate, also ovoid or elliptical, beaked, dark muri form with transverse, longitudinal or even sometimes oblique septa (dictyospores),borne in chains or singly, conidiophores dark, short or elongate, mostly simple or branched

These spores contributed5.08% % to the total air spora.

Aspergillus type :

Conidia of this spore type are globose,1-celled variously colored when enmasse, produced in basipetal succession. The conidiophores developed, that of *Aspergillus* were upright, simple terminating in a globose or elevate vesicle bearing phialides at the apex or they were seen radiating from the whole surface.

The incidence of Aspergillus was dominated with highest percentage of 25.42%. with five species identified as

Table-2. Petri plate Culture Method
Fungal colonies developed on RBS media on Petri Plates-(Indoor Environment of caves)

Sr.No	Fungal species	Total-CFU	%AGE
1	*Absidia* sp.	289	2.61
2	*Acremonium* sp.	354	3.20
3	*Alternaria alternata*	562	**5.08**
4	*Aspergillus flavus*	428	3.87
5	*Aspergillus fumigatus*	621	**5.61**
7	*Aspergillus nidulans*	563	**5.09**
6	*Aspergillus niger*	456	4.12
8	*Aspergillus oryzae*	549	4.96
9	*Botrytis* sp.	232	2.09
10	*Candida* sp.	125	1.13
11	*Chaetomium* sp.	569	**5.14**
12	*Chrysosporium* sp.	254	2.29
13	*Cladosporium herbarum*	1257	**11.36**
23	*Curvularia* sp.	236	2.13
14	*Fusarium solani*	563	5.09
15	*Gliocladium* sp.	231	2.08
16	*Humicola* sp.	122	1.10
17	*Mucor* sp.	423	3.82
18	*Penicillium* sp.	415	3.75
19	*Phoma* sp.	216	1.95
20	*Rhizopus stolonifer*	1896	**17.14**
21	*Stachybotrys* sp.	352	3.18
22	*Trichoderma viride*	345	3.11
		11058	**100%**

Aspergillus flavus :

The surface colony is olive to lime green in colour, 3.87%

Aspergillus fumigatus :

The surface colony is smoky grey to green in colour.It is an Ascomycetes fungus that causes an infection of the lungs called "Aspergillosis". 5.61%

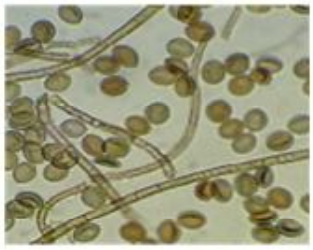
Asperillus nidulans :

The surface colony is dark green or dark olive buff with orange to yellow in areas with cleistothecial formation.5.09%

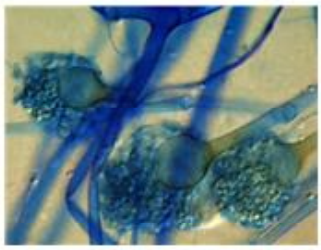
Aspergillus niger :

The surface colony is initially white

Alternaria alternata

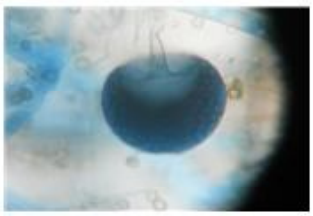


Chaetomium sp.

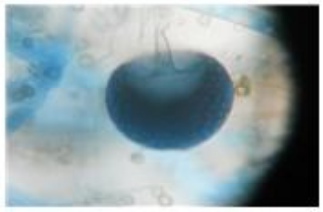


Absidia sp.

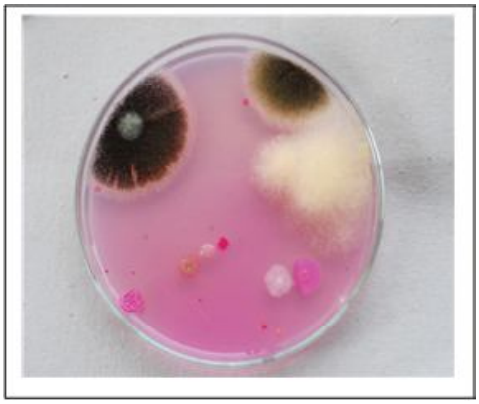
Cladosporium sp.

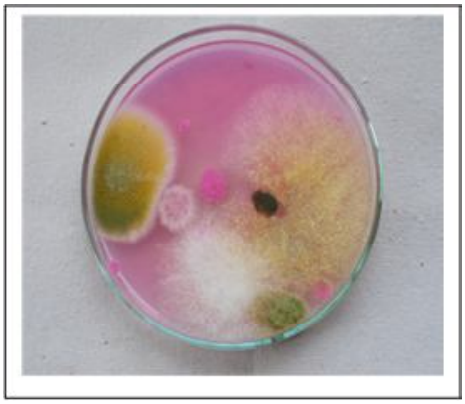


Rhizopus stolonifer



Aspergillus flavus





becoming black to deep brown.4.12%

Aspergillus oryzae :

The surface colony is pale greenish-yellow, olive-yellow or with different shades of green, typically with dull brown shades with age.4.96%

Chaetomium :

The spores are unicellular, dark, circular to triangular or lemon shaped, more or less flattened in one plane.
This fungal spore type contributed 5.14% to the total air spora.

Cladosporium :

The conidia of this fungus are dark,or sub-hyaline. It is one or two celled, polymorphous, ovoid to cylindrical.The conidiophores are dark and shows various type of branching

near the apex,upright single or clustered bearing conidia in simple or branched acropetalchains. This spore was recorded throughout the year with second highest percentage of 11.36 % to the total air spora.

Curvularia :

The conidia are dark,3-5 celled,end cells lighter,more or less fusiform, typicallybent, having one of the central cell enlarged. The conidiophores usually simple, brown, bearing conidia apically or on new sympodial growing points.

This fungal spore type contributed 2.13 % to the total air spora.

Fusarium :

The conidia of Fusarium are hyaline variable mainly of two types-macroconidia and microconidia. The macroconidia are several celled slightly curved or bent at the pointed ends and typically canoe shaped. The microconidia are 1-celled, ovoid or oblong, borne singly or in chains, some of the conidia which are intermediate, 2-3 celled ,oblong or slightly curved.

The spores dominated during rainy season with a percentage of 5.09% to the total air spora.

In the present study 19 fungal spore types were recorded with total of 22 species. *Rhizopus-17.14% Cladosporium-11.36%, Aspergillus fumigatus-5.61%, Chaetomium-5.14% & Aspegillus nidulans-5.09%* were most recorded fungal species in the caves. Most fungal colonies were reported during the months of June to Aug 2019 with increasing humidity during rainy season.

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