RESEARCH ARTICLE

ASSESSMENT OF BIODETERIOGENS OF INTRAMURAL ENVIRONMENT OF KANHERI CAVES

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ABSTRACT

Kanheri caves are a group of rock cut monuments situated at Borivali National park surrounded by deep green forest.Kanheri caves represents Buddhist caves 475 m above sea level. Due to close proximity to urban settlement, these caves are well exposed to anthropogenic activities, pollution and weathering due to biological growth such as fungi, lichens, Algae and Bacteria. The present study focused on assessment of biodeteriogens of intramural Environment of Kanheri caves. The study was carried out for six months fromMarch to Aug 2013.For Petri plate fungal culture Rose Bengal streptomycin media was used .Sampling was carried out once a month. Glycerin jelly coated Slides were exposed every 15 days and observed under the microscope. Fungal spores contributed maximum, Pollen grains and algal hyphae were also encountered during the sampling but not quantified. Miscellaneousbioparticulate matter such as plant parts like trichomes, insect parts were also observed.

Keywords : Caves, Fungal spores, Pollen grains, bioparticulate matter

INTRODUCTION

Kanheri caves are a group of rock cut monuments situated at Borivali National park surrounded by deep green forest. Kanheri caves represents Buddhist caves 475 m above sea level,. Due to close proximity to urban settlement, these caves are well exposed to anthropogenic activities, pollution and weathering due to biological growth such as fungi, lichens, Algae and Bacteria. The airborne particulate matter normally contains a variable percentage of biological particles that may be viable (able to germinate and/or to develop) or not viable. The viable biological particulate is constituted of microorganisms (viruses, bacteria, fungi and their spores), spores of bryophytes and pteridophytes, lichen propagules, algal cells, pollen grains, protozoan cysts.

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In the recent years, the aerobiology has a new field of application: the conservation of cultural heritage. Many organisms and microorganisms able to damage works of art (biodeteriogens) are diffused by air, such as bacteria, fungi, algae, lichens, bryophytes and pteridophytes. The spores or vegetative forms of these biodeteriogens can reach the materials surfaces by deposition and contaminate them. If the cells are sufficiently viable, the environmental conditions (climate and microclimate) and the substrate characteristics are favourable; the biological contaminants can develop and colonize the surfaces. Anthropogenic activities and littering inside the caves also contributes to growth of biodeteriogens.

MATERIAL AND METHODS

A) Gravity slide sampling:

Glycerine jelly coated micro slides were exposed, by using locally fabricated Durham's spore sampler due to its economy and simplicity, inspite of its limitations. The exposures were done at a height of 2 meters, for duration of twice a month consecutive for six month inside the caves. The Glycerine jelly had the following constituents:

Glycerine	-150gm
Gelatin	-50gm
Distilled Water	-150ml
Phenol Crystals	-5gm
And a small trace of saffranin	

For the preparation of glycerine jelly, the desired quantity of gelatin was mixed with distilled water taken in a beaker. It was boiled in a water bath.Glycerine was added to this after about 30 minutes and constantly stirred with a glass rod. The boiling was continued for another $1^{1/2}$hrs, till the mixture became homogenous and translucent without any air bubbles. After removing the beaker from the water bath phenol was added to the mixture and stirred again. Phenol acts as a preservative as well as metabolic inhibitor. A few drops of conc.saffranin were added to the mixture at this stage and the medium was once again mixed thoroughly. The mixture was then poured into sterilized glass vials, covered to avoid contamination, and was stored at room temperature.

For exposure of the slide, a small piece of glycerine jelly was placed over the micro slide and gently heated over the gas flame till it melted. A thin smear of it was made by using another slide drawn over it an angle. Each of these slides after exposure for 24 hrs.was replaced by fresh glycerine jelly coated slide. The exposed slides were placed horizontally in slide boxes and brought to the laboratory for microscopical examination.

A cover slip (18mm X 18mm) was placed over the exposed slide after placing a drop of molten glycerine jelly .The edges of the cover slip were sealed with DPX and after proper labeling the slide was kept horizontally for at least an hour. Scanning of these exposed slides was regularly carried out under high power (10X45) of the Research microscope. A constant quadrate of exposed area of 3.24cm^2 was thoroughly screened for the air-borne micro bio particles.

The pollen grains caught on the exposed slides were identified by morphological features. Reference slides, standard references and illustrations (Nair, 1996) were used for comparative studies leading to their correct identification. Pollen grains were identified on the basis of their size, shape, the type and distribution of apertures and ornamentation pattern of exine. For the identification of fungal spores, their distinct morphological features were marked out with the help of referenced slides and standard illustrations and reference books (Alexopoulos 1962, Barnett and Hunter 1972, Tilak 1989, 1998).

The number of pollen grains, fungal spores and other biological particles were counted accordingly and the results were calculated and arrived at, to give the number per cm2 from the constant exposed area of 3.24cm^2 per slide. The month wise average % contribution of individual spore group to the monthly total air spora was tabulated. This method brought out the qualitative analysis of air-borne microbial-components.

B) 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 2 meters from ground level at the Indoor environment of caves. 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

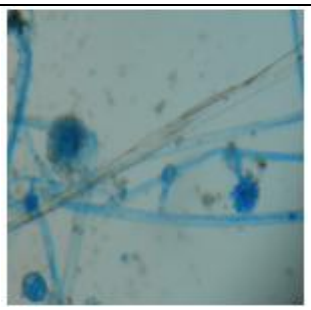
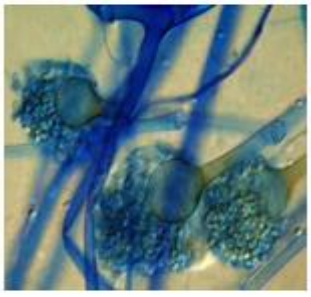
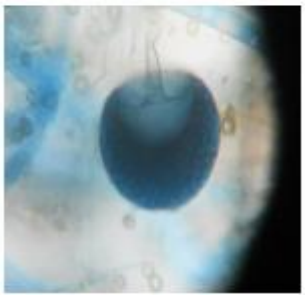
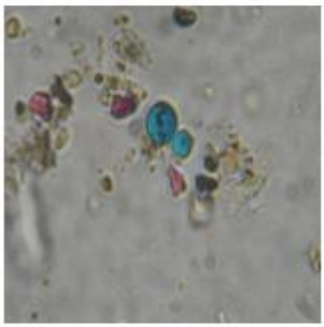
All the above ingredients were mixed in a beaker by adding Distilled water and were boiled in a water bath. It was continuously stirred with a glass rod. Later on it was sterilized by autoclaving at a pressure of 15 lbs. for 20 minutes. Soon after cooling the medium to about 45^{o}c in an incubator, 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 petridishes, 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^{o}c-

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.

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.

RESULTS & DISCUSSION:

Overall in the present study fungal spores were recorded more in the intramural environment. The common fungal spore trapped during the present study from the intramural environment of the caves includes the following: *Absidia sp. Alternaria alternate, Aspergillus flavus, Basidiospores, Chaetomium globosum, Cladosporium sp, Cunninghamella sp, Curvularia sp, Dreschlera sp, Fusarium sp, Penicillium sp, Rhizopus sp, Trichoderma sp.* Pollen grains of grasses were also observed in addition to plant parts, insect parts, Trichomes, etc.

		
Alternaria alternata	***Alternaria alternata***	***Aspergillus flavus***
		
Absidia sp.	***Rhizopus sp.***	***Dreschlera sp.***
		
Grass Pollens	***Lichens***	***Insect parts***

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