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Variations in aerial mycobiota of archeological sites of taxila, Pakistan

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Abstract

The transportation of air borne fungal spores to the surface of archeological monuments is very significant step in the process of biodeterioration. The present study was designed to isolate the aerial mycobiota from six world heritage sites of Taxila. The fungal spores were trapped by petri plate gravitational method and three culture media malt extract agar, potato dextrose agar and czapek dox agar were used. A total of 30 fungal species belonging to 19 different genera were recorded through out the year. The quantitative analysis of data revealed that *Alternaria alternata* with 9.79% of total colonies was the dominant species in the air of selected sites followed by *Aspergillus niger* (9.10%), *Cladosporium herbarum* (8.02%), *Penicillium chrysogenum* (7.53%), *Fusarium oxysporum* (6.94%), *Aspergillus* flavus (6.73%), *Aspergillus fumigatus* (6.0%), *Penicillium frequentans* (4.68%), *Cladosporium cladosporioides* (3.85%), *Alternaria solani* (3.78%), *Mucor mucedo* (3.50%) and *Helminthosporium solani* (3.40%). The qualitative analysis of isolated fungal species clearly indicated a well marked variation in the composition of aerial mycobiota of selected sites as some fungal species were restricted to particular archaeological sites. The present investigation is first study of aerial mycobiota of world fame archaeological sites of Taxila.

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Introduction

In recent few years it has been found by many researchers that microbes as biodeteriogens can damage a variety of cultural materials. The colonization of microorganisms on building materials of stone monuments and their mechanism of decay is generally linked with climatic and environmental conditions around the monuments. The fungal spores in air and soil play more dangerous role in biodeterioration of archeological monuments because fungal spores are always dominant in air and soil (Pandey, 1988). The successful colonization of air borne fungal spores on archaeological monuments depends upon several climatic and environmental factors along with composition of substrates (Nugari, 2003). Therefore, the study of different aspects of aeromycobiota especially the composition of fungal spores of a particular site could be very useful to prevent the establishment of fungal spores on the surfaces of archeological monuments.

Some aero mycological studies have been conducted in different parts of the world to monitor the role of air borne fungi in biodeterioration of monuments and historical buildings. In India (Pandey *et al*., 2011) isolated the fungal species of *Alternaria ,Aspergillus, Beauveria ,Bipolaris , Curvularia , Cochliobolus , Cladosporium , Chaetomium, Crysosporium , Conidiobolus , Drechslera , Exserohilum ,Fusarium ,Penicillium , Sepedonium , Scopulariopsis , Trichothecium , Torula* and *Ulocladium* from air of Gwalior fort. Aira *et al*.,(2007) conducted an aero mycological study of cathedral of Santiago Compostela(Spain) by using viable volumetric method. They isolated 35 different fungal species. Alternaria, Aspergillus, Cladosporium and Penicillium were found dominant genera in their findings. The fungal genera *Alternaria, Aspergillus* and *Dreschlera* were prevalent in the air of different sites of Rohtas fort in Pakistan (Shah and Bashir, 2008).They also revealed that *Aspergillus niger* with 24.08% was found as dominant fungal species. The composition of air and soil borne fungal spores as contaminants of stone in hypogean cemetery in Slovak republic was calculated by Simonovicova *et al* in 2004.They isolated *Acremonium strictum, Alternaria alternata, Aspergillus vesicolor, Auerobasidium pulleurans, Cladosporium specie, Penicillium chrysogenum, Penicillium viridicatum* and *Trichoderma* specie in their investigation.

The variation in air borne fungi isolated from different historical and non historical sites was also an evident in the findings of many works. Many fungal species were found dominant in the composition of aerial mycobiota of different environments. In Italy Urzi *et al* (2001) studied that *Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium, Ulocladium, Auerobasidium* and *Phoma* were the most dominant fungal species in the air of Messina Museum. They also found that the presence of these fungal spores is a cause of biopitting and formation of black patinas. In a non historical place Nicoleta and Dorina (2009) in Romania isolated Cladosporium, Setosphaeria, Alternaria and Epicocum as dominant fungal species along with other air borne fungi while Anna and Anna and Marinella (2000) conducted a research to isolate air borne fungi from air of two under ground station in Milan, Italy. They found that *Cladosporium, Penicillium, Epicocum* and *Alternaria* were common fungal genera.

The transportation of fungal spores from air and their settlement on surface of monuments is very important as fungi can cause the spoilage of building materials through their physical and chemical activities. (Burford *et al*., 2003). The hyphae of fungi can penetrate deeply in to the constituents of stone and other materials resulting in biopitting and cracking (Crook and Burtan, 2010). The presence of fungal spores in air around archeological sites is an indicator of future decay of monuments because many fungal spores start growing on the surface of monuments under suitable conditions (Sterflinger, 2010). These fungal spores by secreting metabolites, enzymes and organic acids cause colored stains, patinas and also cause transformation of many

mineral which result in loss of materials (Maria *et al*., 1999; Gadd and Sayer, 2000; Martino *et al*., 2003). Fungi can also cause biodeterioration of harder material of monuments as many fungal species have been identified as significant biodeteriogens even on quarry and concrete (zherebyateva *et al*, 1991; Bock and Sand, 1993).

The world fame archaeological sites of Taxila, Pakistan are under threat of biodeterioration especially microbial decay of stone monuments is very common. The present study was designed to evaluate the status of aerial mycobiota of world heritage sites of Taxila to understand their roles in the process of biodeterioration.

Materials and methods

Sampling Sites

The archeological sites of Taxila are world fame for Buddhist monasteries, stupas, chapels and figural decorations. Six world heritage sites including Bhir mound, Dharmarajika, Sirkap, Mohra Moradu and Jaulian were selected for present investigation. The stone monuments of these selected archaeological sites have large number of biofilms, stains and patinas as a sign of biodeterioration.

Sampling routine and technique

The isolation of aerial mycobiota from selected archeological sites was done by petri plate gravitational method (open plate method) previous used by Asan *et al*., 2002; Uddin, 2004 with efficient results. In order to isolate maximum fungal spores three culture media Potato dextrose agar, Mart extract agar and Czapek dox agar were used. The media containing plates were exposed for 10 minutes at a height of 1m by holding in hands. These Petri plates were then sealed with paraffin and wrapped in Aluminum foil before transferring to laboratory.

Four sampling points were selected at each site to cover all directions and maximum area. Four Petri plates were exposed at each point. Three Petri plates contained separate media i.e. MEA, PDA and czapek dox agar while a Petri plate containing MEA was served as control. The Petri plates containing the samples were incubated for 3 to 5 days at room temperature (25 to 28°C).

Identification of Mycoflora

The fungal species were identified by study of detailed taxonomic features and keys by (Cooke, 1963), Nilson (1983) and Domsch *et al*., (2007). The morphological and microscopic feature of each colony was noted and determinations of morphological structures of fungi were carried out after being mounted in lacto phenol and cotton blue covered with cover slip. The fungi were identified up to genus level and in some cases up to species level. Each colony was further isolated and their pure cultures were maintained on MEA.

Results and discussion

Composition of aerial Mycobiota

A total of 2879 fungal colonies were calculated from the entire air sample obtained from 6 archeological sites of Taxila. The composition of aerial mycobiota was consisted of 30 fungal species belonging to 19 different genera. The composition of aerial mycobiota of archeological sites of Taxila is given in table 1.

Table 1. Composition of aerial mycobiota of archaeological sites of Taxila.

Serial Number	Fungal genera	Fungal Species
01	*Acremonium*	*Acremonium sp*
02	*Alternaria*	*Alternaria alternata , Alternaria solani, Alternaria brassicae*
03	*Arthobotrys*	*Arthobotrys sp*
04	*Aspergillus*	*Aspergillus candidus, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus terrus*
05	*Cladosporium*	*Cladosporium herbarum, Cladosporium cladosporioides*
06	*Cochliobolus*	*Cochliobolus specifer*
07	*Curvularia*	*Curvularia lunata*
08	*Dematium*	*Dematium sp*
09	*Epicocum*	*Epicocum purpurascens*

Serial Number	Fungal genera	Fungal Species
10	*Fusarium*	*Fusarium oxysporum, Fusarium culmorum*
11	*Geotrichum*	*Geotrichum candidum*
12	*Helminthosporium*	*Helminthosporium solani*
13	*Mucor*	*Mucor mucedo, Mucor hiemalis*
14	*Penicillium*	*Penicillium chrysogenum, Penicillium frequentans*
15	*Phoma*	*Phoma glomerata*
16	*Rhizopus*	*Rhizopus oryzae, Rhizopus stolonifer*
17	*Trichocladium*	*Trichocladium asperum*
18	*Trichoderma*	*Trichoderma sp*
19	*Trichothecium*	*Trichothecium sp*
Total	19	30

Quantitative analysis of Aerial mycobiota

The results of present investigation showed that *Alternaria alternata* was pre dominant fungal in the air of archeological sites of Taxila. The annual total of fungal colonies and their percentage of occurrence are given in table 2. *Alternaria Alternata* 9.79% of total colonies was the dominant fungal species followed by *Aspergillus niger* (9.10%), *Cladosporium Herbarum* (8.02%), *Penicillium chrysogenum* (7.53%), *Fusarium oxysporum* (6.94%), *Aspergillus flavus* (6.17%), *Aspergillus fumigatus* (6.0%), *Penicillium frequentans* (4.68%), *Cladosporium cladosporioides* (3.85%), *Alternaria Solani* (3.78%), *Mucor mucedo* (3.50%), *Helminthosporium solani* (3.40%), *Mucor hiemalis* (3.26%), *Rhizopus oryzae* (3.09%) and *Curvularia lunata* (3.02%).

Table 2. Quantitative analysis of fungal colonies isolated during investigation period.

Fungal Species	Bhir mound	Dharma-rajika	Sirkap	Sirsukh	Jaulian	Mohra Moradu	Total	% of total colonies
Acremonium sp	7	15	13	0	0	16	51	1.77
Alternaria alternata	45	73	28	19	65	52	282	9.79
Alternaria solani	17	32	7	9	25	19	109	3.78
Alternaria brassicae	0	6	0	0	0	0	6	0.20
Arthobotrys sp	3	5	3	0	0	0	11	0.38
Aspergillus candidus	11	20	10	0	0	7	48	1.66
Aspergillus fumigatus	26	49	16	13	39	30	173	6.00
Aspergillus flavus	27	54	17	21	43	32	194	6.73
Aspergillus Niger	40	70	22	26	60	44	262	9.10
Aspergillus terrus	0	2	0	0	3	0	5	0.17
Cladosporium herbarum	35	65	18	24	54	35	231	8.02
Cladosporium cladosporioides	17	32	7	11	26	18	111	3.85
Cochliobolus specifer	33	9	16	0	0	0	58	2.01
Curvularia lunata	12	24	19	0	19	13	87	3.02
Dematium sp.	10	13	13	6	7	6	55	1.91
Epicocum Purpurascens	0	13	5	0	0	0	18	0.62
Fusarium Oxysporum	28	54	16	24	46	32	200	6.94
Fusarium culmorum	0	25	19	0	18	0	62	2.15
Geotrichum sp	0	13	5	0	4	0	22	0.76
Helminthosporium solani	16	30	6	7	22	17	98	3.40
Mucor mucedo	27	46	2	0	0	0	101	3.50
Mucor hiemalis	16	24	7	15	19	13	94	3.26
Penicillium chrysogenum	32	63	15	21	50	36	217	7.53
Penicillium frequentans	21	42	9	14	25	24	135	4.68
Phoma glomerata	0	0	5	4	0	18	27	0.93
Rhizopus oryzae	21	38	8	0	0	22	89	3.09
Rhizopus Stolonifer	4	17	5	9	10	7	52	1.80
Trichoderma sp	20	18	0	0	0	0	38	1.31
Trichocladium asperum	0	0	19	10	0	0	29	1.00
Trichothecium sp	3	9	0	0	0	2	14	0.48
Total	471	861	336	233	535	443	2879	

The isolated fungi from the air of archeological sites of Taxila with low concentration were *Fusarium culmorum* (2.15%), *Cochliobolus specifer* (2.01%), *Dematium sp.* (1.91%), *Rhizopus stolonifer* (1.80%), *Acremonium sp.* (1.77%), *Aspergillus candidus* (1.66%), *Trichoderma sp.* (1.31%), *Trichocladium asperum* (1.0 %), *Phoma glomerata* (0.93%), *Geotrichum sp.* (0.76%), Epicocum *purpurascens* (0.62%), *Trichothecium sp.* (0.48%), *Arthobotrys sp.* (0.38%), *Alternaria brassicae*(0.20%) and *Aspergillus terrus* (0.17%).

At genus level *Aspergillus* was found as most dominant genus with 5 species (682 colonies with 23.68%), followed by *Alternaria* with 3 species (397 colonies, 13.78%, *Penicillium* with 2 species (352 colonies, 12.15%), *Cladosporium* with 2 species (342 with11.89%), *Fusarium* with 2 species (262 colonies, 9.10%), *Mucor* with 2 species (195 colonies, 6.77%) and *Rhizopus* with 2 species (141 colonies with 4.89%).

The results of present investigation showed that air around the monuments of Taxila is contaminated with large number of fungal spores. Many fungal spores isolated in present study were also encountered by many researchers from the air of different monumental sites in the world.

The fungal genera *Alternata, Aspergillus, Cladosporium* and *Penicillin* were dominant in the air of architectural complex of the cathedral of Santiago de Compostela (spain) (Aira *et al., 2007).* These fungal genera were also dominant in present investigation while Maggi *et al.,* 2000 found *Alternaria, Aspergillus* and *Chaetonium* as commonly found genera in state archives of Rome. The air of Messina Museum was found contaminated with *Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium, Ulocladium, Auerobasidium* and *Phoma.* In china Woufu (2010) isolated *Cladosporium, Penicillium, Alternaria* and *Aspergillus* as most prevalent fungal genera in open and close caves.

Qualitative analysis of aerial mycobiota

A well marked variation in the composition of aerial mycobiota of 6 selected archeological sites of Taxila was found in present investigation. Many fungal species were found restricted to a particular site. The variation in the composition of aerial mycobiota is given in table 3.

Table 3. Comparative analysis of aerial mycobiota of archaeological sites of Taxila.

Fungi	Bhir mound	Dharm arajika	Sirkap	Sirsukh	Jaulian	Mohra Moradu
Acremonium sp	+	+	+	-	-	+
Alternaria alternata	+	+	+	+	+	+
Alternaria solani	+	+	+	+	+	+
Alternaria brassicae	-	+	-	-	-	-
Arthobotrys sp	+	+	+	-	-	-
Aspergillus candidus	+	+	+	-	-	+
Aspergillus fumigatus	+	+	+	+	+	+
Aspergillus Flavus	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	+	+
Aspergillus terrus	-	+	-	-	+	-
Cladosporium herbarum	+	+	+	+	+	+
Cladosporium cladosporioides	+	+	+	+	+	+
Cochliobolus specifer	+	+	+	-	-	-
Curvularia lunata	+	+	+	-	+	+
Dematium sp.	+	+	+	+	+	+
Epicocum Purpurascens	-	+	+	-	-	-
Fusarium Oxysporum	+	+	+	+	+	+
Fusarium culmorum	-	+	+	-	+	-
Geotrichum sp	-	+	+	-	+	-
Helminthosporium solani	+	+	+	+	+	+
Mucor mucedo	+	+	+	-	-	-
Mucor hiemalis	+	+	+	+	+	+

Fungi	Bhir mound	Dharm arajika	Sirkap	Sirsukh	Jaulian	Mohra Moradu
Penicillium chrysogenum	+	+	+	+	+	+
Penicillium frequentans	+	+	+	+	+	+
Phoma glomerata	-	-	+	+	-	+
Rhizopus oryzae	+	+	+	-	-	+
Rhizopus Stolonifer	+	+	+	+	+	+
Trichoderma sp	+	+	-	-	-	-
Trichocladium asperum	-	-	+	+	-	-
Trichothecium sp	+	+	-	-	-	+

The fungal species *Alternaria Alternata*, *Alternaria solani*, *Aspergillus fumigatus*, *Aspergillus niger*, Aspergillus flavus, *Cladosporium herbarum*, *Cladosporium sporiodes*, *Dematium sp*., *Fusarium oxysporum, Helminthosporium solani, Mucor hiemalis, Penicillium chrysogenum*, *Penicillium frequentans* and *Rhizopus Stolonifer* were detected as commonly found fungal species in the air of all selected archeological sites of Taxila.

Some variations in the composition of aerial mycobiota of individual sites were recorded in present findings. Maximum fungal species were isolated from Dharmarajika (28 species) followed by Sirkap (26 species), Bhirmound (23 species), Mohra Moradu (20 species), Jaulian (18 species) and Sirsukh (16 species). Some fungal species were totally absent from a particular archaeological site. Out of thirty fungal species only *Phoma glomerata* and *Trichocladium asperum* were not recorded in the air of Dharmarajika through out the investigation period.

The fungal species *Alternaria brassicae, Aspergillus terrus, Epicocum purpurascens, Fusarium culmorum and Geotrichum sp*. were not encountered in the air of Sirsukh, Bhirmound and Mohra moradu while *Trichocladium asperum* was only recoded in the air of Sirkap similarly *Trichoderma* was only isolated from Dharmarajika and Bhirmound. Some other variations in the occurrence of air borne fungal spores were also an evident in present investigation.

The variations in quality and quantity of air borne fungal spores in archeological sites and non archeological areas may be due to many factors. The climatic and environmental factors along with the presence of waste materials around a particular area could affect the composition of aerial mycobiota.

In comparative studies of aerial mycobiota many researchers found that some species were dominant and commonly found in their findings. Masghazy *et al.,* (2012) studied indoor Aeromycoflora of monumental sites of Minia Governorate in Egypt. They isolated 56 fungal species belonging to 28 genera from 45 places. The major components of Aeromycoflora were *Aspergillus* (10 species), *Penicillium* (5 species), *Alternaria* (3 species), *Cladosporium* (2 species), *Mucor* (3 species), *Ulocladium* (3 species) and *Phoma* (1 specie). El. Hissy *et al*., (1991) isolated *Alternaria Alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terrus, Cladosporium sp., Fusarium sp., Mucor sp., Penicillium sp., Rhizopus* and *Ulocladium sp*. were identified from samples collected from Dandara Temple (Qena) and Abidos Temple (Sohag).

The present investigation clearly indicated that the air of selected archeological sites of Taxila is contaminated with a large no of fungal spores and many of these spores have been reported as deteriorative agents in bio-deterioration of many world fame archeological monuments and historical buildings around the world. The monitoring of these fungal spores is very important to avoid any threat of decay and deterioration of world fame stone monuments of Taxila.

Conclusion

The results of present study indicated that the presence of some important fungal species around the

air of monumental sites of Taxila could be a source of Bio-deterioration of precious monuments of Pakistan. Conservation authorities should take the serious notice about the future existence of archeological monuments.

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