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SEASONAL VARIATIONS IN THE AEROBIOLOGICAL PARAMETERS OF A STATE ARCHIVAL REPOSITORY IN INDIA

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ABSTRACT

There is the need for detailed studies of degradative microbial agents affecting archival materials in tropical countries like India. It has been observed in earlier studies that environment plays a key role in the preservation of archives. The State Archives under consideration is one of the biggest collections in the world. A seasonal quantitative as well as a qualitative study of microbial aerosols in the archival repository indicated a perennial prevalence of cellulolytic fungi as well as bacteria within the repository. Their existence could be well correlated to the geographical location of the archives and hence its environmental conditions. The qualitative analysis in terms of the isolates' cellulolytic, proteolytic and amylolytic activities were indicative of their degradative abilities. Study revealed that the predominant fungal genera in the repository belonged to *Aspergillus*, *Cladosporium* and *Penicillium*. With 1000 CFU m^{-3} as a permissible limit for indoor air quality, a heterotopic count for fungi equating to 14.8 x 10^3 CFU m^{-3} and that of bacteria to 52.2 x 10^3 CFU m^{-3} in autumn presses the need to arrest the numbers so as to preserve the documents as well as to prevent any occupational hazards caused by these bioaerosols.

KEYWORDS: Air micro-flora, archives, indoor air quality, environmental parameters.

INTRODUCTION

The Maharashtra State Archives department or The Directorate of Archives which is headquartered in Mumbai, India holds one of the world's largest collection of archives numbering over 30 crore documents, some dating back to the 16th century. India is a tropical country and the rate of deterioration of manuscripts is greatly affected by the environmental

factors like heat, humidity, salinity due to the proximity to the sea (Attri & Tyagi, 2010). Predominantly high tropical temperatures and humidity are crucial factors progressing the rate of degradation, as well as providing an agreeable atmosphere to various pests and insects (Lin et al. 2007; Havlínová et al., 2009; Stephens & Whitmore, 2013). The high moisture content typically found in tropical countries aids in bio-chemical degradation, there by accelerating deterioration of the documents. Increase in both heat and humidity provides an ideal environment for insect, bacterial and fungal attacks respectively. The tropics are, in fact, about as far as one could get from any vision of an ideal library or museum environment (Cappitelli & Sorlini, 2005; Majumdar, 2005; Bankole, 2010; Borrego et al., 2010). Mumbai's climate can be best described as moderately hot with high level of humidity across all seasons. It's coastal nature and tropical location ensures a stable range of temperatures without much fluctuations through the year. Mumbai experiences four seasons: Winter (Dec-Feb); Summer (March-May); Monsoon (June–Sept) and Post-Monsoon-Autumn (Oct-Nov).

As environmental microorganisms are capable of deteriorating archives and other archival material, it is important to investigate the microbial concentration of indoor air at repositories (archives and libraries) so as to design effective methods of preservation of the cultural heritage. Possible sources of biological contamination of indoor air include: influx of people, dust arising from cleaning/dusting and various materials stored in the repositories, and the inflowing air from the ventilation and air conditioning systems (Niesler et al., 2010; Kalwasinska et al. 2012;).

Due to their specific functional character, archival repositories constitute a unique micro-environment where the possibility of air contamination with microorganisms developing on the damp documents is high. When favorable microclimatic conditions occur, the microorganisms are likely to infect the library collections and initiate the process of biodeterioration. Damage to paper is primarily due to fungal species belonging to the genera *Aspergillus, Trichoderma, Penicillium, Alternaria, Cladosporium* and *Rhizopus,* and, to a lesser degree, to heterotrophic and cellulolytic bacterial species like *Cellulomonas, Bacillus, Cellfalciculata, Cellvibrio* and *Cytophaga* (Allsopp et al., 2004; Sterflinger, 2010; Sequeira et al. 2012; Sterflinger & Pinzari, 2012).

Additionally, these microorganisms are also known to affect the general health of people who work in the premises or handle these resources. The findings of epidemiological research indicate that exposure to high concentrations of microbes in the air frequently leads to

allergies, asthma, hay fever (Bjornsson et al. 1995; Newson et al. 2000) pneumonia (Siersted & Gravesen, 1993), and many other side-effects, including infections (Ren et al. 2001). Biological factors such as fungal spores and mites are involved in sick building syndrome, a complex situation in which occupants experience a variety of symptoms and become generally unwell, recovering only when they cease to frequent the building (Dales et al. 1991; Ross et al. 2000; Newson et al. 2000).

Most of microorganisms that exist at indoor environments are saprophytic and obtain nutrients for their metabolism from inorganic and organic material such as wood, paper, painting, dust etc. (Florian, 2004; Valentín, 2010). Paper being cellulose based, acts as a microhabitat for a number of microorganisms. They use it as a growth stratum as well as a food source. The additives and manufacturing components used in paper such as glues, bindings, inks, etc. supplement the growth of these microbes as they are rich in organic carbon (Michaelsen et al. 2010). In addition to organic carbon, the very hygroscopic nature of paper supplements the proliferation of microorganisms especially fungi (Pinzari et al., 2006; Valentín, 2010). When considering paper stored in archival repositories, its colonization and biodegradation depends on composition of the air microflora predominantly the cellulolytic organisms, since they are the ones that initiate colonization on the bulk of the substrate.

The aims of this research were to study microbial air quality and the environmental conditions and its possible influence on biodeterioration of materials stored in the archives (Maharashtra State Archives), Mumbai India.

MATERIALS AND METHODS

Location

The Directorate of Archives (Maharashtra State Archives), is housed in the Elphinstone College in South Mumbai; Latitude: 18.930153 Longitude: 72.831356 within 3 Kms of vicinity to the sea (Fig.1). Their archival collection is housed in eight stack rooms having total area of about 30,000 sq.ft. The records are arranged on 120 iron racks each about 17 feet in height, occupying an area of approximately 94,000 cubic feet. The racks have 12,760 compartments and each compartment holds 25 to 40 volumes depending on the size of the records.

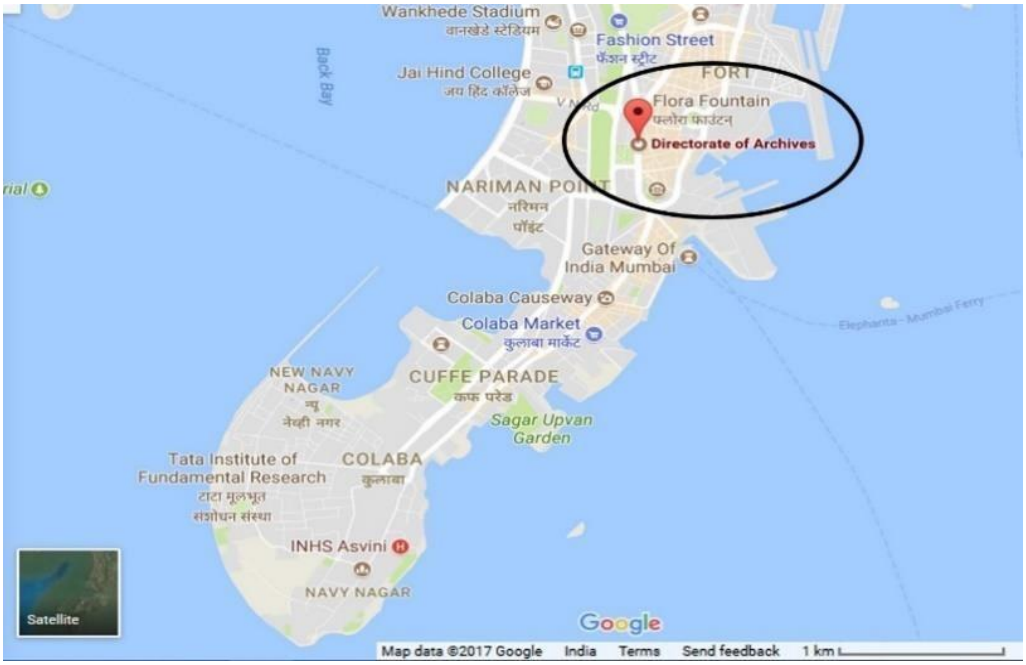

Fig 1: Location of The Directorate of Archives on satellite map (Google maps, 2017).

Environmental factors

The values for temperature, wind, weather, relative humidity was obtained from the Indian Meteorological department, an agency of the Ministry of Earth Sciences of the Government of India. It is the principal agency responsible for meteorological observations, weather forecasting and seismology.

Microbiological sampling of air

Four different sampling areas of department of Archives were selected based on the following criteria: (1) Types of archival documents stored i.e. *Daptars*, Dairies, Maps, Newspapers (2) storage space availability (3) the exposure of the storage area to sunlight (4) availability of ventilation. These areas demarcated were sampled throughout the four seasons for a year. Microbiological sampling was carried out by the sedimentation method as described by Omeliansky (Bogomolova & Kirtsideli; 2009, Guiamet; 2011). Passive sampling provides a valid risk assessment as it samples the causative air flora in the airborne population which could sediment onto a surface of the archives. Petri dishes containing Sabouraud Dextrose agar (SAB) for the isolation of fungi and Nutrient agar for (NA) bacteria were exposed for 15 mins at specified locations.

The sampling was conducted during mid-afternoon i.e. between 11 am-1 pm a time period during which the archival department sees a maximum influx of people. All the samples so

obtained were incubated for 24-72 hrs at room temperature (RT) (28-30°C). The CFU per cubic meters of air ($CFUm^{-3}$) was estimated according to Omeliansky's formula:

$$N = 5a \times 10^{4}(bt)^{-1}$$

Where, N = microbial $CFUm^{-3}$ of indoor air, a = number of colonies per Petri dish, b = dish surface, in square centimeters, and t = exposure time, in minutes.

The colonies so obtained were screened for cellulolytic activity using Carboxy Methyl Cellulose (CMC) congo-red agar (Huang; 2012). Cellulose degrading fungal and bacterial colonies, showing discoloration of Congo-Red on the solid medium were identified and characterized using (Lu 2004; Gupta 2012) cultural, morphological and biochemical characteristics (Ellis 2007).

Assessment of biodegradative abilities by plate assays

Protease activity

To assess the proteolytic activity, the isolated microflora was grown on milk-nutrient agar (Milk-NA), which was prepared by adding (25 ml) sterile skimmed milk to sterile Nutrient Agar (75 ml). The plates were then incubated at RT for 24-48 hrs. After incubation, the zone clearance was indicative of the enzymatic activity.

Amylase activity

The isolates were tested for amylase activity by employing zone clearing technique Atlas et al. (2004) using starch agar medium. The inoculated plates were incubated at RT for 24- 48 hrs. After incubation, the zone of hydrolysis of starch was detected by flooding the plates with iodine solution. The development of blue color indicated the presence of starch, while the areas around the hydrolytic organisms appeared clear.

RESULTS AND DISCUSSION

Environmental conditions

The temperature, relative humidity (RH), and wind speed monitored during the study is as documented in table 1. As is typical for a costal tropical location, the temp was found to range between a min of 27°C during winters to a max of 34°C in summer. The relative humidity was consistently above 55% irrespective of the seasons and the wind velocity above an average of 15 km/hr. due to the proximity (2-3 Kms) of the archives to the sea. Geographical location and climate play a critical role in determining type as well as the load microflora in the environment. The most significant environmental factors influencing the

viability of aero flora are temperature, relative humidity and wind speed (Jones 2004; Mouli 2005;2006; Mota 2008). The counts of bacteria and fungi are strongly affected by these factors as they thrive & propagate under specific environmental conditions (Jones 2004). While temperature and water availability affect the source and particularly aid the release of fungal spores in the atmosphere, the wind speed helps in creating mechanical disturbance or strong air movement sufficient to disrupt materials and cause aerosolization (Mandal 2011).

Table 1: **Seasonal variations in the environmental parameters.**

Months	Temp	Weather	Wind	Humidity
Summer				
March –May	31-34 °C	Passing clouds. Clear sky	15-19 km/h	59-67%
Monsoon				
June-September	27-31 °C	Rain. More clouds than sun.	17-20 km/h	79-94%
Autumn				
October- November	30-32 °C	Scattered clouds.	11-13 km/h	63-75%
Winter				
December-February	27-30 °C	Scattered clouds. Clear sky	13-15 km/h	28-59%

The prevalence of fungi and bacteria (in terms of $CFUm^{-3}$) in air at the archives are as shown in Fig. 2. Data indicated a high prevalence for both bacteria and fungi in the indoor environment. The total airborne fungi and bacteria monitored showed a perennial pattern of occurrence. An increase in autumn (August & November), winters (January) and peak in early monsoon (June) during the year-long monitoring period is recorded.

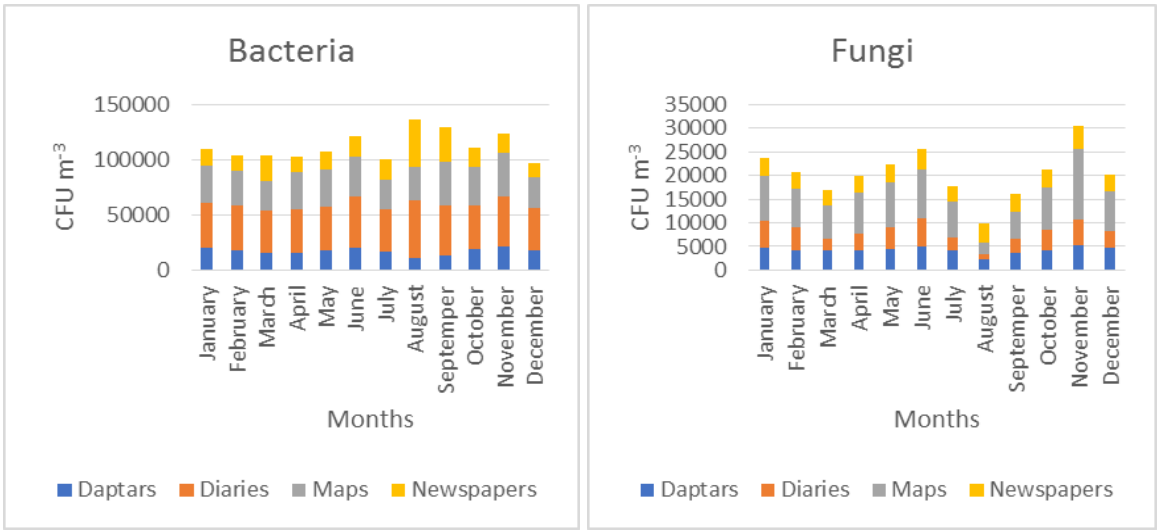

Fig. 2: Bacterial and fungal distribution ($CFU\ m^{-3}$) throughout the year.

Metal racks have been used to house the maps, which have been rolled and stored one of top of the other. Besides this the presence of wooden cupboards, sitting areas for the employees

was notable. The area was however well ventilated (cross ventilation) and received indirect sunlight through a close by window. Dairies have been kept in conditions, without cross ventilation, however receiving indirect sunlight. They have been neatly arranged in the storage racks unlike the maps. *Daptars* and newspapers were stored in areas which similar to those of the maps i.e. with sitting arrangements, presence of wooden cupboards, however devoid of ventilation as well as natural light. The area was illuminated with artificial lights. The predominantly higher fungal prevalence in the areas of the maps throughout the year except in the month of august can be attributed to the ventilation as well as the wind blowing across the open windows, bringing in the fungal load from the outdoor environment. In august / autumn the fungal count in all the four areas has been comparatively lower due to the sedimentation caused due to heavy rains. January and November showed highest fungal count of 9.6 x 10^3 CFU m^{-3} and 14.8 x 10^3 CFU m^{-3} in the area of maps compared to August where it dropped to a lowest count of 1.1 x 10^3 to 4 x 10^3 CFU m^{-3}. Bacteria showed maximum prevalence in the areas where diaries were stored followed by the area where maps are kept. The month of august shows highest bacterial count 52.2 x 10^3 CFU m^{-3} (diaries) and lowest in December i.e. 38.4 x 10^3 CFU m^{-3} (diaries). Though highest bacterial and fungal counts were obtained in autumn (Aug and Nov) the total microbial load was the highest in the month of May followed by October and February. The fungal concentrations in the archives department was extremely high throughout the year with a small dip in the month of august especially in the area maps were kept. All the four months had the bioaerosol count of more than 1.5 x 10^5 CFU m^{-3}. The relative humidity and temperature in moderate range along with high wind speed as noted in the environmental factors, might have facilitated the release and dispersion of dry spore mass; which presumably contributed towards achieving the peak during these months. The proximity of the archival department to the sea as well as its vicinity to a very busy street, favors the influx of large amount of dust carrying fungal spores and bacteria and other particulate matter. The microbial prevalence in the air of the archives was very high in comparison to studies previously performed (particularly for bacteria) (Borrego et al. 2010; Niesler et al., 2010; Anaya et al. 2016; Kadaifciler, 2017). Eagle Industrial Hygiene Associates (2004) and Wonder Makers Environmental, Inc., 2001, organizations working for environmental, health and safety have suggested that environments with a microbial prevalence above 1000 CFU m^{-3} should be considered contaminated. Guidelines for indoor air quality in offices in Brazil consider that 750 CFU m^{-3} should be the lower limit for air-micro flora (Radler de Aquino and de Góes, 2000). On comparing the data

collected in this study the indoor air quality of the archives department seems to be highly compromised.

Fig. 3 depicts the results of the preliminary identification carried out to assess the biodegradation potential of the microbes in the archival environment. *Cladosporium* spp. (26%) prevailed the most in the air followed by *Penicillium* spp. (21%) *Aspergillus* spp. (20%). *Fusarium* spp., *Rhizopus* spp., *Alternaria* spp., *Trichoderma* spp. and *Neurospora* spp. were prevailed at comparatively lower percentages (8-3%).

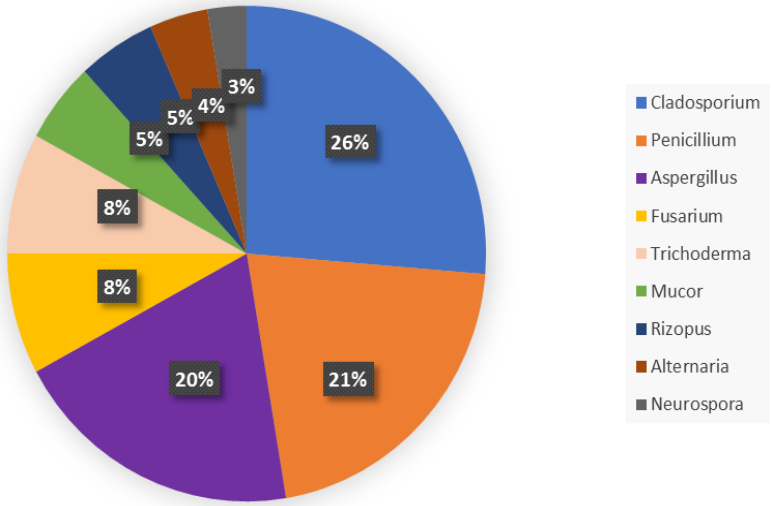

Fig. 3: Fungal concentrations throughout the year.

The composition and species variability of airborne fungi observed in the study corroborated with earlier reports in India (Vittal, 1981; Adhikari, 2004). In this study, *Cladosporium* spp. was recorded as the prevalent airborne fungal genus irrespective of the season of sampling. With respect to the bacterial isolates from air, gram-positive bacteria were the most abundant at the repository. Gram-positive bacteria represented about 74% of total bacterial isolates. On the basis of primary identification; biochemical tests, *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Streptomyces*, and *Bacillus* genera identified, have been consistent with other studies (Guiamet, 2007).

The biodegradative abilities of the selected organisms are as described in the table 2. Biodeteriogens found on papers act through the cellulolytic enzymes dissolving cellulose fibers in papers resulting in rapid loss of strength and disfigurement of archival materials. Proteases secreted by microorganisms break down other materials on paper, like fillers or sizing agents, rich in proteins like gelatin. Amylases are secreted by the microorganisms in

response to starch that is generally used for surface sizing as well as for coatings. The deterioration caused by these enzymes and also by the secreted organic acids, cause a gradual loss of mechanical strength in paper (Abdel-Kareem, 2010; Valentin, 2010) finally leading to disintegration and permanent loss of information. These microbial byproducts of degradation are not only harmful to the archival material but also to those handling the material for purposes of referencing or its conservation. Majority of the fungal strains isolated from the air of archives, libraries, and museums exhibit cellulolytic, proteolytic, and/or amylolytic activities; produce acids; excrete different pigments; and contribute to the formation of biofilms, which accelerate the deterioration of the different document substrates (Ren, 2001; Rafał, 2002; Florian, 2004; Guiamet, 2007; Nevalainen, 2009; Borrego, 2010; Kalwasińska, 2012; Jacob, 2015).

Table 2: Biodegradative abilities of the isolated air flora.

Organism	Cellulase	Protease	Amylase
Cladosporium isolate 1	+++	+	++
Cladosporium isolate 2	+++	++	++
Cladosporium isolate 3	+++	+	+
Cladosporium isolate 4	++	++	+++
Penicillium isolate 1	+++	++	++
Penicillium isolate 2	+++	++	++
Penicillium isolate 3	++	+++	+++
Penicillium isolate 4	+++	+++	++
Aspergillus isolate 1	+++	++	+++
Aspergillus isolate 2	+++	+++	+++
Aspergillus isolate 3	+++	++	++
Aspergillus isolate 4	++	+++	+
Aspergillus isolate 5	+++	+	++
Fusarium isolate 1	+++	++	+
Fusarium isolate 2	+++	+++	++
Fusarium isolate 3	+	++	++
Trichoderma isolate 1	+++	++	++
Trichoderma isolate 2	++	++	+
Trichoderma isolate 3	+++	+	+++
Mucor isolate 1	+++	+	++
Mucor isolate 2	+++	+	+
Rizopus isolate 1	+++	++	++
Rizopus isolate 2	+++	+	++
Alternaria isolate 1	+++	+++	++
Alternaria isolate 2	++	++	+++
Neurospora isolate 1	++	+	+
Bacillus isolate 1	++	+++	++
Bacillus isolate 2	+++	++	+++
Bacillus isolate 3	+++	+	++

Bacillus isolate 4	+++	++	+++
Bacillus isolate 5	+++	++	++
Bacillus isolate 6	+	++	+++
Bacillus isolate 7	+++	++	+
Bacillus isolate 8	++	+++	+++
Staphylococcus isolate 1	+	++	-
Staphylococcus isolate 2	+	+	+
Staphylococcus isolate 3	+	++	-
Streptococcus isolate 1	+	+	++
Streptococcus isolate 2	+	+	+
Micrococcus isolate 1	+	++	++
Micrococcus isolate 2	+	++	+
Streptomyces isolate 1	+++	+++	++
Streptomyces isolate 2	++	+++	++

Key: + : mild ++ : moderate +++ : high

Primary colonizers of archival deterioration like *Penicillium* and *Aspergillus* can grow at low water activity (aw) values (aw < 0.8). Whereas other fungal genera (e.g. *Alternaria* and *Cladosporium*) require aw values at least between 0.8 and 0.9 (Nielsen, 2003). A high relative humidity throughout the year justifies the prevalence of all of these three species throughout all the seasons. These fungi are reported to be the commonly found isolates in the archival environment libraries, as well as museums (Ren, 2001; Florian, 2004; Borrego, 2010; Jacob, 2015, Skora 2015).

Our observation that Gram-positive bacteria prevailed among bacterial isolates at the archives is consistent with other studies (Guiamet 2007; Borrego 2010). *Staphylococcus* and *Streptococcus* are primary microbial flora of skin and mucous membranes. Some species of *Staphylococcus*, *Streptococcus*, and *Bacillus* are potential or opportunistic pathogens. Since *Bacillus* and *Streptomyces* species are usually cellulolytic they constitute a potential risk for papers. High concentration of bacteria even if they aren't cellulolytic is also alarming as these organisms can initiate secondary degradation post the infestation by some cellulolytic fungi.

Many common environmental fungi produce secondary metabolites that are toxic to eukaryotic cells. *Cladosporium*, *Penicillium* and *Aspergillus* are capable of producing various mycotoxins leading to the sick building syndrome. Spores or bacteria can lead to respiratory ailments as they easily get trapped within alveolar lung tissues. Continued exposure (through inhalation) to bacteria producing endotoxins is known to cause irritation of the respiratory system, resulting in shivers, nausea, fever, malaise, and headache, bronchitis and asthma. These organisms are also known to result in skin allergies and infections.

As a remedial measure, it is ideally recommended that heating, ventilation and air conditioning systems be designed indoors to prevent the entry of bio-aerosols and to maintain conditions within a closed environment to deter microbial growth. One of the primary problem at the Maharashtra state archives is the maintenance of temperature and humidity between 18 to 22°C and 55% respectively. These conditions make the repositories very vulnerable to micro and macro organisms which can thrive well. Air-conditioned libraries instead of naturally ventilated buildings have been suggested by Singh et al. (1995) since they have lower fungal spore concentrations in the archival environment. In some tropical countries the daily usage of air conditioners can be difficult due to costs involved and the intermittent power supply. In a case of intermittent power supply air conditioners would prove to more deleterious than useful due to condensation. In such conditions, alternative means of air circulation like fans could be installed. These would help to eliminate areas of high moisture in spaces in stacks and archival collections. It is well known that an environment with fluctuations in temperature and humidity level is the most harmful as materials expand and contract in such conditions resulting in their weakening. Archival documents should be kept in dust free enclosures as mold spores are carried as well as settled through dust and another suspended particulate matter. The present aerosol sampling of an important archival repository in India is one of the first studies that signifies the necessity to control environmental factors as an immediate step to archival preservation.

CONCLUSION

This study highlights a significantly high culturable air borne microbial load in an important State Archives in India. Fungal genera belonging to *Aspergillus*, *Cladosporium*, *Penicillium*, *Alternaria*, *Fusarium* isolated, exhibited biodegradative abilities which are hazardous for the archival material. These are also known to be potential allergens, toxic, and opportunistic fungal pathogens. It is this of utmost importance to control the levels of bioaerosols in the repository. Most tropical countries possess a greater amount of atmospheric humidity, the incidence of humidity and temperature also provide an ideal breeding ground to micro and macro organisms, which further ravage these timeless artifacts. Regular cleaning and efficient ventilation could aid in improving the air quality and arresting the deterioration of the archival documents.

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