Int. J. Mendel, Vol. 31 (3-4), 119-123, 2014 **INTRAMURAL AEROBIOLOGY**

BIODETERIOGENS AND ALLERGENS IN THE AMBIENT AIR OF BHARATI PRINTING PRESS, PUNE

Jogdand Sudam Baburao

Key words : Biodeteriogens, allergens, ambient air, Printing Press.

It is now a proven fact that biodeterioration of paper materials in the press, library, etc., and human allergy are caused by a number of airborne microbes, usually paraphrased as *biodeteriogens* and *allergens* respectively. Hence, aerobiological investigations have been carried to find out the load of different biodeteriogens and bioallergens in the intramural environment of the Printing Press of Y.M. College, Pune from January to April, 2012 in relation to meteorological parameters using Tilak Continous Air Sampler. Interesting results have been recorded. Similarly significant effects of environmental factors on these aeromicrobes have been observed. Altogether 101 aerobiocomponents belonging to different groups of fungi, animal parts and other types have been recorded. Deuteromycotina have been found to dominate all other groups and *Cladosporium* has been recorded as the dominant type.

INTRODUCTION

Pune is the largest city of Maharashtra situated in the Western Ghats of India. It has a tropical wet and dry climate with average temperature ranging between 20 to 28°C (68 to 82°F). Continuous air sampling was carried out by electrically operated Tilak Air Sampler in the Printing Press of Bharati Vidyapeeth Deemed University, Erandwane, Pune.

Usually paper materials are damaged by various microbes in paper industry, bookstalls, library, godown and printing press. Hence indoor aerobiological study was carried out in the Bharati Printing Press using Tilak Air Sampler to analyse the general aerospora and to find out the relevance of some biocomponents in the biodeterioration of binding materials, paper, books and incitation of health problems like allergy, etc., among workers in the printing press.

Many of these spores are biodeteriogens causing biodeterioration of materials in the press while some of them are allergens responsible for causing allergy among the sensitive press workers. These spore types showed monthly variations and diurnal fluctuations with varying spore load in the air of printing press.

The number of fungal spore types and their diversity vary with day time, weather, season, geographical location and the presence of local sources. The highest number of airborne spores was found to be in temperate and tropical regions and the least in the desert (Lacey, 1981). There is impact of aerobiocomponents on plants, animals and human beings (Agarwal *et al*., 1969). Jacobs (1951) elaborated the term aerospora to include dispersion of airborne insect populations, fungal spores, pollen and bacteria. Since fungal spores have long been known as one of the important environmental bio-components causing dermatitis, respiratory and cardiac diseases along with allergic manifestations in human beings; therefore, preliminary study on airborne fungi has been conducted in the printing press of Pune.

Fungal propagules can serve as infective agents of plant diseases and, moreover, airborne fungi cause spoilage of foods and are responsible for many adverse health effects; the mycotoxins which they produce may affect humans and animals.

MATERIALS AND METHODS

Continuous air sampling was carried out from January, 2012 to April, 2012 by electrically operated Tilak Air Sampler in the printing press of Bharati Vidyapeeth Deemed University, Erandwane, Pune to analyse the general aerospora and to find out their biodeteriogenic and allergenic relevance.

Collection of Data : The cellotape was fixed over the rotating drum of Tilak Air Sampler. The spore catches of air sampling on loaded tape coated with petroleum jelly were collected weekly. The loaded tape on the rotating drum was cut into 14 equal pieces, 4.2 cm each and mounted separately using melted glycerin jelly to prepare permanent slides. These slides were labelled with dates, month and year and day/night.

Scanning : Loaded tape on each slide was divided into six equal divisions by marking it over cover slip with a pointed ball pen,each division representing two hours spore catches. Scanning of marked slides in sequence was carried out under the binocular research microscope, using 10x X45x magnification, as per the procedure mentioned by Tilak(1987). The identification of fungal spore types was made on the basis of size, colour, shape, septation, structure and morphology of spores using standard keys and authentic available literature.

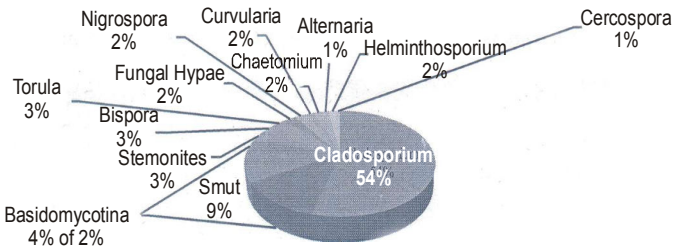
Statistical Analysis : To obtain volumetric concentration, total spores count/day were multiplied by conversion factor 14 of Tilak Air Sampler. Monthly total and monthwise aerospora was calculated as usual.

Results and Discussion : This study revealed incidence of 101 spore types and other components in the environment of the press. Some of the dominant spores in the descending order are *Cladosporium* (47.22%), *Aspergillus* (10.87%), *Smut* (7.97%), Basidiospores (3.41%), while the spores like *Lophiostoma* (0.0086%), *Harknesia* (0.02%) and *Botryodiplodia* (0.025%) were found minimum. The dominant spore types observed were of *Cladosporium* (54%) followed by *Aspergillus* (12%), Smut (9%), *Basidiospores* (3%),

Assoc. Scientist, Yashwantrao Mohite College, BVDU, Pune-411 038, Maharashtra, India
E-mail : sudam.jogdand@gmail.com

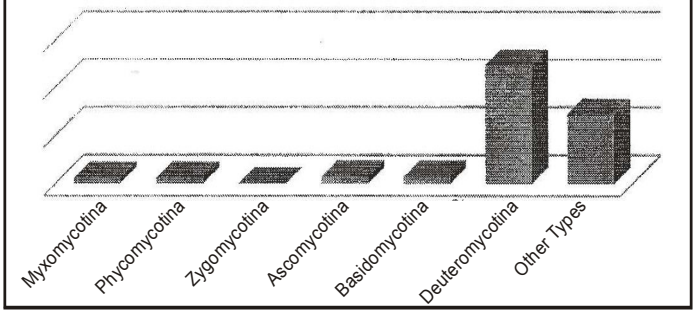
Stemonites (2.98%), *Torula* (3%) and *Bispora* (3%), Fungal Hyphae (2.11%), *Nigrospora* (2.06%), *Curvularia* (1.503%), *Chaetomium (1.41%), Alternaria (1.I5%), Helminthosporium* (1.27%) and *Cercospora* (0.76%). As per these results it was observed that *Cladosporium* was dominant and *Pithomyces* was lowest. All these biodeteriogens have been found responsible for the damage of papers and other cellulytic materials in the library as shown in the photograph.

Graph No. 1 : Percentage contribution of prominent spore types to the total aerospora (2012) in the intramural environment of Bharati Printing Press, Pune



Percentage contribution of prominent spore types in the intramural environment of Bharati Printing Press to the total aerospora revealed the highest percentage contribution of *Cladosporium* (54%), followed by *Aspergillus* (12%), Smut (9%), Basidiospores (3%), *Stemonites* (3%), *Torula* (3%) and *Bispora* (3%) and minimum percentage contribution of *Chaetomium* (l.41 %), *Alternaria* (1.15%), *Helminthosporium* (1.27%) and *Cercospora* (0.76%).

Graph. No. 2. : Groupwise percentage contribution of aerobiodeteriogens in the intramural environment of Bharati Printing Press, Pune

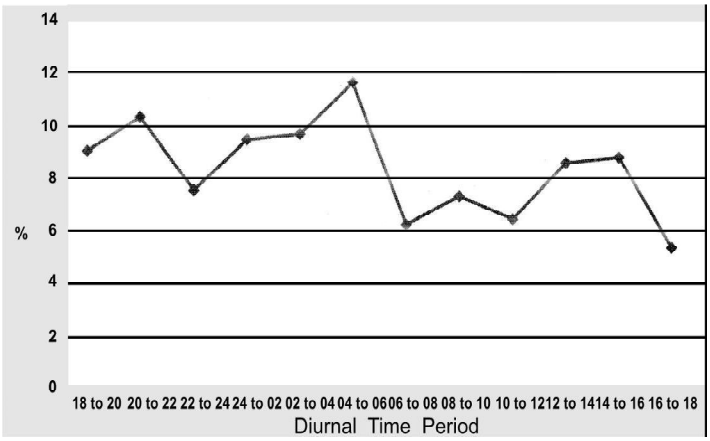


Groupwise percentage contribution of aerospora in the intramural envronment of Bharati Printing Press at Pune from January 2012 to April 2012 revealed Deuteromycotina to be dominant (49.07%) followed by other types (28.62%) over Basidiomycotina (16.84%). However, Ascomycotina (2.85%) and Myxomycotina (2.27%) were encountered in very less concentration and Zygomycotina (0.047%) and Phycomycotina (0.34%), were encountered in minimum percentage concentration.

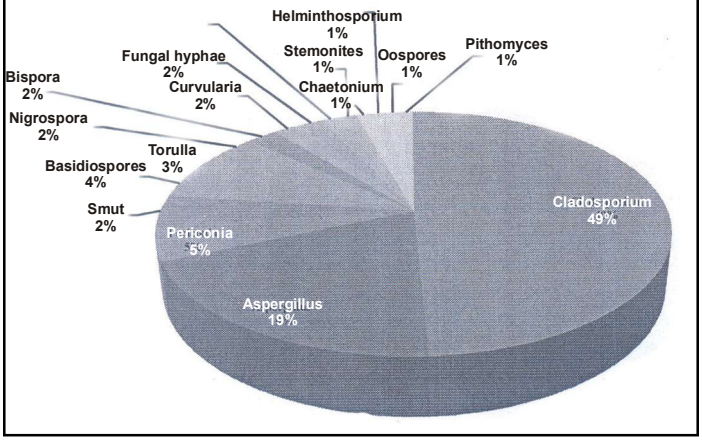
Diurnal periodicity curve of the prominent spore like *Cladosporium* in the intramural environment of Bharati Printing Press revealed peak between 8.00 a.m. and 10.00 a.m. during day time just before noon followed by subsidiary between 10 a.m. and 12 noon and minimum at 06 a.m. to 08 a.m. and 14-16 hrs.

Diurnal periodicity curve of *Nigrospora* in the intramural environment of printing press, Y. M. College, Pune from 11-20 Jan, 2013 recorded main peak between 4 a.m. and 6 a.m. and subsidiary peak between 20 and 22 hours, thus representing “Night Spora Group” and minimum between 16 and 18 hrs. (Fig 1).

Fig. 1 : Diurnal periodicity curve of average percentage contribution of *Nigrospora* from 11-20 Jan, 2013 to the total aerospora of that week in printing press.

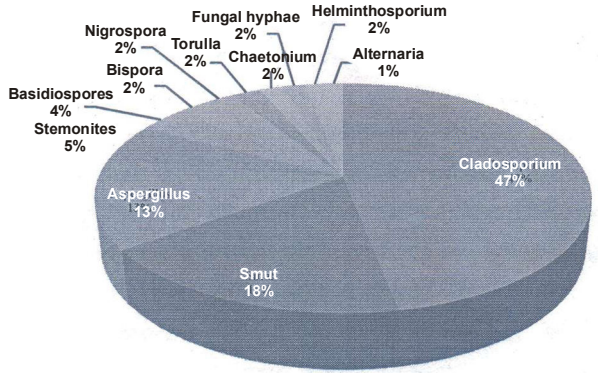


Graph No. 3 : Percentage contribution of biodeteriogens and prominent spores in the air sampling of Bharati Printing Press, Pune in January 2012



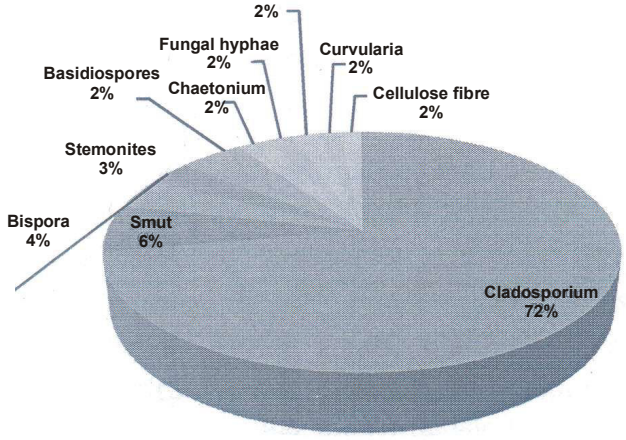
In the month of January, 2012, out of 74 spores types, *Cladosporium* was dominant (51896) followed by *Aspergillus* (20398) and *Periconia* (4760) while *Pithomyces* (1050) revealed moderate percentage contribution during Jan 2012 in the air of Bharati Printing Press.

Graph No.4. Percentage contribution of prominent biodeteriogens in February 2012 in Bharati Printing Press, Pune



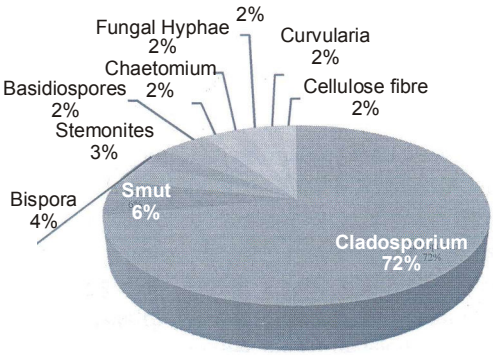
In the month of February, a total of 95 spores were observed. The abundant spores found in February were *Cladosporium* (31968), *Aspergillus* (6272), Smut (5058), Basidiospore (3498), *Stemonites* (3262), Fungal hyphae (1960), *Torula* (1526), *Bispora* (1442), *Curvularia* (1162), *Alternaria* (1078) and *Hysterium* (1078). As the results of February month reveal that *Cladosporium* was the highest spore followed by *Aspergillus,* Smut, Basidiospores, *Stemonites,* Fungal hyphae, *Torula, Bispora, Alternaria* and *Hysterium* while *Sirosdesmium*, *Pseudotorula* and *Beltraneilla* were found to be least. *Othia, Sporodesmium, Hirudinaria* and *Spicaria* have also been reported.

Graph No.5. : Percentage contribution of Prominent biodeteriogens of March 2012 in Bharati Printing Press, Pune.



Total 44 different spore types were observed during the month of March, 2012. *Cladosporium* was found to be major and abundant spore (36206), followed by Smut (13804), *Aspergillus* (9630), *Stemonites* (4126), Basidiospores (2748), *Bispora* (1904), *Torula* (1763), *Cercospora* (957) and *Curvularia (798).*

Graph No. 6. : Percentage contribution of Prominent biodeteriogens in April 2012 in Bharati Printing Press, Pune



In the month of April 2012, out of 53 spores types, *Cladosporium* was dominant (37617), followed by *Aspergillus* (5706), Smut (3360), *Alternaria* (2226), *Bispora* (2080), *Nigrospora* (1680), *Stemonites* (1274) and *Chaetomium* (1220) which revealed moderate percentage contribution during April 2012 in the air of Bharati Printing Press, Pune. *Clasterosporium*, *Passereneilla* and *Rosellina* were the least spore types found in this month.

DISCUSSION :

Present aerobiological investigation in the intramural environment Bharati Printing Press revealed 101 spore types dominated by *Cladosporium*. Many of them are found to be biodeteriogens while some of them are known aeroallergens. In several aeromycological studies, *Cladosporium* was considered as one of the most, if not the most, abundant spore types reported all over the world (Lacey,1981; El-Essawy *et al*. ,1992, Shaheen, 1992; El-Said and Abdel-Hafez,1995; Ismail *et al*.,2002; Al-Subai, 2002; Asan *et al*.,2004; Hedayati *et al*., 2005; Oliveira *et al.,* 2005; Nourian *et al*.,2007; Ozkara *et al*.,2007). According to Shaheen (1992), the abundance of *Cladosporium* throughout the year may be attributed to the structural features of the spores such as small size, thin exine and smooth wall which favour and facilitate the transport of airborne spores. Other workers have indicated that *Cladosporium* spores are the dominant airspora in hot climates (Takahashi, 1997; Sen and Asan, 2001; Al-Subai, 2002; El-Morsy, 2006). Myszkowska *et al*. (2002) noted that some 4-7% of the European population shows sensitivity *to Alternaria* and *Cladosporium spores.* C. *cladosporioides,* the most common reported species, has been the agent causing phaeohyphomycosis along with other species of *Cladosporium* (Kantarcioglu *et al*. 2002). These results coincide with our observations.

A variation in the temperature, humidity and rainfall was noted during the investigation period. *Cladosporium* species thrives as a saprophyte or parasite on many kinds of plants. Dry spores produced in excessive quantities can be transported over wide areas and during rainy season its concentration was low (Ebner *et al*., 1989). Spores of

Cladosporium were most common in the air due to wet and warm season and maximum spore count wa noted in the month of February. However, Ballero *et al.,* 1984; Ebner *et al.*, 1989; Halwagy, 1989 and Hjelmroos, 1993 reported increased atmospheric concentration of *Cladosporium* after rainfall with long lasting peak, some hours after the rain. Mitakakis *et al.,* 1997 reported a negative correlation with rain.

In the present study *Aspergillus* has been found to be dominant fungal spore type (10.87%) in the intramural environment of Bharati Printing Press at Pune, causing health problems in some of the workers of Printing Press. *Aspergillus nigeris* has been found well known to cause many health hazards such as extrinsical veolitis, allergic bronchopulmonary aspergillosis, keratitis, endophthalmitis, primary cutaneous aspergillosis and necrotising otitis in humans (Severo *et al.* ,1997). In addition to being aeroallergens, the above fungi may produce mycotoxins or other fungal metabolites that are carried by airborne dust and reach humans through skin contact (Sarica *et al.*,2002).

In this study, *Alternaria, Chaetomium, Mucor, Curvularia, Pithomyces,* etc., are of regular occurrence and major biodeteriogens causing damage to paper material. In some European countries, *Alternaria* varies between 20,000-30,000 spores/year; however, in the Iberian Peninsula the annual representation varies from close to 70,000 spores/year (Oliveira *et al.*, 2007) to more than 200,000, only exceeding the levels of 300,000 spores quoted for the north-western Iberian Peninsula in some areas (Mediavilla *et al.*,1997). On the contrary, the levels of *Alternaria* spores, with maximum mean annual levels of 2417 spores as indicated in this study, are clearly surpassed by those from other centres, in excess of 15,000 spores (Damialis and Gioulekas, 2006). As in other parts of Europe, the concentration of spores in the north-western Iberian Peninsula increases during the summer. In several Italian cities, high quantities of *Cladosporium* and *Alternaria* are found from May to October, reaching their maximum levels in September (Zanca, 2003). However, in areas at lower latitudes where precipitation and humidity are limiting factors, but not temperature, the spores increase in the months before and after summer (Manoharachary *et al.*, 2005) Our observations coincide with above observations to a certain extent and are contrasting to some limits.

The various biodeteriogens obtained during the study have been found to cause biodeterioration of papers, fabrics, binding materials, threads, gums, books, etc., especially during rainy season when these goods are stored uncared in damp places.

Comparative investigations revealed clear picture of monthly, seasonal and annual variation with respect to total types obtained and total load encountered during these months, seasons or years respectively giving clue for the preparation of seasonal and annual spore calendars of these bioallergens or biodeteriogens.

Number of spore types obtained during 2013 have been recorded to be more during seven months as compared to that of 2012 and less during remaining 05 months. While quantitative estimation has revealed more load in first 09 months, i.e., from January to September during 2013 as compared to that of 2012 and less load during remaining 03 months i.e., Oct., Nov. and Dec. during 2013. It has been recorded highest during August (2,52,168) 2013 followed by July 2013 (2,26,268) and lowest during May (12,236) followed by April 2012 (27,340). Maximum number of spore types or qualitative estimation of these two consecutive years has been recorded during September 2013 (62) followed by August 2013 (61) and minimum during April 2012 (27) followed by May 2012 (33).

Classwise fluctuations in dominant class Deuteromycotina during two consecutive years with respect to total number of spore types revealed maximum biodiversity during September 2013 (33 types) followed by Jan., Feb., Aug. and Dec.2013, i. e., 32 types in each while minimum during April (17) and May (19) during 2012 followed by Nov. (20) during 2013. Quantitative estimation revealed maximum load during January (1,01,444) followed by Aug. (97,062) 2013 and Jan. (60,816) followed by Aug. (58,324) during 2012 while lowest during May (7,672) 2012 followed by Dec. 2013 (18,606).

Qualitative observations from Ascomycotina revealed highest biodiversity during September 2013 (18) followed by Aug. 2012 and 2013 (17 each) and minimum during April (04) followed by May (05), while quantitative estimation revealed highest load during Aug. (95,998) followed by June (81,739) during 2013 and lowest during April (574) followed by May (588).

This study revealed the existence of a rich population of airborne fungi in the atmosphere of the intramural environment of Pune in the Bharati Printing Press, which varied in species composition, abundance and monthly fluctuation. The 101 spore types trapped in the study belonged to different divisions like Myxomycotina, Phycomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina. Deuteromycotina dominated over other divisions. Many of these have been found to be biodeteriogens causing damage to the press materials and some of them have been found to cause allergy in sensitive individuals. Thus this study bears significance from the point of view of biodeterioration and allergy.

References

Agarwal, M.K., Mukharjee, K.G. and Shivpuri, D.N. 1969. Studies on the allergenic fungal spores of Delhi, Indian Metropolitan Area, Botanical Aspects J. Allergy, **44**: 193-203.

Al-Subai, A. A., 2002. Air-borne fungi at Doha, Qatar. Aerobiologia, **18** (3-4), 175-183.

Asan, A., Ilhan, S., Sen, B., Erkara, I., Filik, C., Cabuk, A.,*et al.,* 2004. Airborne fungi and actinomycetes concentrationsin the air of Eskisehir City (Turkey). Indoor Built Environment, **13**: 63-74.

Ballero, M., Gionnis, N.D., Goretti, G., Lombartdibi, S. and Frenquwlli, G.,1984. Compararative study about airborn spores in Cagliari and Perugia. Aerobiologia, **8**:141-147.

Damialis A, Gioulekas, D., 2006 : Airborne allergenic fungal spores and meteorological factors in Greece: Forecasting possibilities. Grana, **45**, 122-129.

Ebner, M.R., Haselwandter, K. and Frank, A., 1989. Seasonal fluctuations of airborn fungal allergens. Mycol. Res. **92**: 170-176.

El-Essawy, A. A., Abd El-Kader, M. I. A., Abou El-Hawa,M.E., & Aly, A. S. E., 1992. Studies on mycoflora of air of Sana'a governorate "Yemen Republic". Egyptian Journalof Applied Science, **7**: 607-616.

El-Morsy, E.S., 2006. Preliminary survey of indoor and outdoor airborne microfungi at coastal buildings in Egypt Aerobiologia, **22**: 197-210.

El-Said, A. H. and Abdel-Hafez, S.I., 1995.Seasonal variations of airborne fungi above banana fields in Qena, Upper Egypt Cryptogamie. Mycologist, **16**: 101-109.

Halwagy, M.H., 1989. Seasonal airospora at the three sites in Kuwait Mycol, Res. **93** :208-213.

Hedayati, M., Mayahi, S., Aghili, R. and Gohm:imoghadam, K. 2005. Airborne fungi in indoor and outdoor of asthmatic patients' home, living in the city of Sari. Iranian Journal of Allergy, Asthma, and Immunology, **4** (4): 189-191.

Hjelmroos, M.,1993. Relationship between airborne fungal spore presence and weather variables, *Cladosporium* and *Alternaria*. Grana **32**: 40-47.

Ismail, M. A., Abdel-Hafez, S. I. 1. and Moharram, A. M., 2002. Aeromycobiota of western desert of Egypt African Journal of Science and Technology, **3** (1): 1-9.

Jacob, W.C., 1951. Aerobiology in the compendium of meteorology. American Meterological Society, Boston.: 1103-1111.

Kantarcio'glu, A. S., Yucel, A. and Hoog, G. S., 2002. Isolation of *Cladosporium* cladosporioides from cerebrospinal fluid. Mycoses Case Report, **45** (11-12): 500-503.

Lacey, J., 1981. Aerobiology of conidial fungi. In G. T.Cole and B. Kendrick (Eds:), Biology of conidial fungi, 1: 273-416.

Manharachary C, Sridhar K, Singh R, Adholeya A, Suryanarayana TS, Rawat S, Johri B. N., 2005. Fungal biodiversity: distribution, conservation and prospecting of fungi from India. Current Science. **89**: 58-71.

Mediavilla A, Angulo J, Infante F, Dominguez E, 1997. Influence of meteorological factors on the incidence of *Cladosporium* Link ex Fr. Conidia in the atmosphere of Cordoba (Spain). Phenology in Seasonal Climates, 1: 117-126.

Mitakakis, T. Ong, E.K., Stevens, A., Guest, D., Knox, R.B. 1997. Incidence of *Cladosporium, Alternaria* and total fungal spores' in the atmosphere of Melbourne (Australia) over 3 years. Aerobiologia, **13**: 83-90.

Myszkowska, D., Stepalska, D., Krystyna, K. and Porebski, G., 2002.The relationship between airborne pollen and fungal spore concentrations and seasonal pollen allergy symptoms in Cracow in 1997-1999.Aerobiologia, **18**: 153-161.

Nourian, A. A., Badali, H., Khodaverdi, M., Hamzehei, H. and Mohseni, S., 2007. Airborne mycoflora of Zanjan-Iran. International Journal of Agriculture & Biology, **9** (4): 628-630.

Oliveira M., Ribeiro H., Abreu I., 2005. Annual variation of fungal spores in atmosphere of proto, 2003. Annals of Agricultural and environmental medicine. **12** (2): 309-315.

Oliveira M, Abreu I, Ribeiro H, Delgado L, 2007. Fungal spores in the atmosphere in the city of Oporto and its allergological implications. Rev Port Imunoalergol **15**, 61-85.

Ozkara, A., Ocak, I., Korean, S. and Konuk, M., 2007. Determination of fungal air spora in Afyonkarahisar, Turkey. Mycotaxon, **102**: 199-202.

Sarica, S., Asan, A.,Tatman-Otkun, M., & Ture, M., 2002. Monitoring indoor airborne fungi and bacteria in the different areas of Trakya University Hospital (Edirne-Turkey). Indoor Built Environment, **11**: 285-292.

Shaneen, I., 1992. Aeromycology of Amman area. Jordon, Grana, **31**: 223-228.

Sen, B. and Asan, A., 2001. Airborne fungi on vegetable growing areas of Edirne, Turkey. Aerobiologia, **17**(1): 69-75.

Severo, L. C., Geyer, G. R., Porto, N. S., Wagner, M. B., & Londero, A. T., 1997. Pulmonary Aspergilli using intracavitary colonization. Report of 23 cases and a review of the literature. **14**: 104-110.

Takahashi, T., 1997. Airborne fungal colony-forming units in outdoor and indoor environments in Yokohama, Japan. Mycopathologia, **139**: 23-33.

Tilak, S.T., 1987. Air Monitoring (Practical Manual), Vaijayanti Prakashan, Aurangabad. pp.110.

Zanca, M., 2003. Aerobiological survey on Deuteromycetes from 1999 to 2002 of Mantua's area (Po Valley-North Italy). In: Emberlin *et al.* (Eds): Proceedings Third European Symposium in Aerobiology. Worcester, 30 August 2 September 2003. University College, Worcester 2003.