

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.506.020>

A Comparative Study of Aeromycoflora in Traffic and Residential Areas of Haridwar City, India

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ABSTRACT

Keywords

Aeromycoflora,
Indoor and
Outdoor
environment,
Relative humidity,
Fungal spores.

Article Info

Accepted:
11 May 2016
Available Online:
10 June 2016

A comparative study of the aeromycoflora in traffic and residential areas of Haridwar City were studied during January- March 2014. The present work was based on the collection of fungal spores from the atmospheric air from the spore trapper technique. Potato Dextrose media was used for the present study. For air sampling, exposed Petri Plate method was used. The investigation showed variations in number as well as in the composition of fungal species of these areas. The maximum average value of colony forming unit of fungal colonies after plate exposure was recorded as 92.5 c/m³ from site II and its minimum average value was recorded as 60.75 c/m³ from site III. The study revealed that the fungal spores were the major components of the air micro flora of both the outdoor and indoor environment of Haridwar city. A total of 13 types of fungi were recognized from both the outdoor and indoor environment and there were some species which could not be identified. *Aspergillus* and *Penicillium* were reported as dominant species of the outdoor environment where as *Cladosporium* was dominant species followed by *Rhizopus* and *Mucor* at residential areas. Other genera like *Alternaria*, *Fusarium*, *Helminthosporium*, were frequently recorded from both the cross-section circles of the city where as *Trichoderma* was the least dominant species occurred at both the outdoor and indoor environment.

Introduction

Air is one of the most important abiotic environmental component and essential for the existence of life, (Joshi, 1995). Air pollution in India is a serious issue with the major sources being fuel wood and biomass burning, fuel adulteration, vehicle emission and traffic congestion. Traffic congestion is severe in India in cities and towns, (Awasthi, 2008). The atmosphere contains all the major groups of microbes

ranging from the viruses to fungi, (Waggoner, 1960). Fungal spores, commonly called mold spores, are a normal component of the outdoor air. The spores are discharged from fungi growing as saprophytes (existing on dead or decaying organic matter in the soil or elsewhere in the environment) or parasites (infecting living tissues), (Levetin *et al.*, 2004). Anytime, moisture or even high humidity is available, spores can germinate and fungi can grow

and produce thousands of new spores, (Levetin, 1995). Air borne fungal spore occur throughout almost the whole year, but the seasonal rhythm in the occurrence of air borne spores and their spectrum depends on the type of climate (Adhikari *et al.*, 2004). The fungal densities in the air also vary in accordance with geographical regions and seasons, besides the physical parameters such as wind direction, humidity, temperature, precipitation and altitude. The fungal species such as *Penicillium*, *Aspergillus*, *Mucor*, *Fusarium* and *Cladosporium* are the dominant fungi in the atmospheric air, (Das and Bhattacharya, 2008). High temperature and the absence of rain are unfavourable conditions for the growth of fungus.

Many fungal spores are allergic capable of causing allergenic responses in susceptible individuals. Epidemiological studies showed that too high concentration of microorganisms in the air can be allergic, however even very low concentrations of some particular microorganisms can cause serious diseases. Human exposure to air-borne micro-organisms may result in a variety of adverse health effects including infectious diseases like legionellosis, Eczema, Onchomycosis, Otomycosis, allergic and irritant responses respiratory problems and hyper sensitivity reactions, (Chandel, 2002). Keeping these facts in view, the present study was designed to evaluate the relative occurrence of certain air borne mycofloral proportions of four different sites in Haridwar city pertaining to different conditions i.e. residential and traffic cross section circles of the city.

Material and Methods

Study Area

The present study was conducted at four different sites in Haridwar district located in

the state of Uttarakhand, two sites of traffic area with Site I (Singh Dwar) and Site II (Jatwara Pul) with heavy pollution load and two sites of residential area with Site III (Vishnu Garden Colony) and Site IV (Gyan Lok Colony) with light pollution load. The study was conducted during winter season for twelve weeks i.e., from January 2014 to March 2014.

Standard procedures were followed in analyzing the samples as prescribed by Aneja, 2003; Watanbe, 2002.

Procedure for Sampling

For air sampling exposed Petri plate method was used. The method of trapping spores by Petri-plates exposure involves the principle of sedimentation under the force of gravity. Four Petri plates (10cm diameter) of freshly poured potato dextrose agar medium were exposed at each site for 10 minutes in afternoon at an interval of 10 days. Plates were exposed at one meter height from ground level at both the sides. Temperature and relative humidity were also recorded with the help of thermometer and hygrometer respectively at the time of exposure. After exposure, the plates were incubated at 28±° C for 3-4 days in incubator.

Calculations

After incubation, the colonies on plates were counted with the help of electronic colony counter.

C.F.U. = Total number of colonies

$$\text{Total Occurrence} = \frac{\text{No. of Colonies of Individual Species in all Plate Studied}}{\text{Total No. of Colonies of all the Species}} \times 100$$

The microscopic examination of fungi was done by using Agar squash mount method given in (Watanbe, 2002).

Results and Discussion

The present work was based on the collection of fungal spores from the atmospheric air by spore trapping technique. This work was carried out at four different locations from residential and traffic cross section circles of Haridwar city. The results of present study are given in Table I, Table II and Table III. A total of 13 fungal species were identified viz. *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Mortierella*, *Mucor*, *Nigrospora*, *Penicillium*, *Rhizopus* and *Trichoderma*. Some species were unidentified.

The mean temperature and mean relative humidity of the atmosphere from the month of January to March are given in Table I. The mean temperature of the atmosphere was increased from 11.9°C to 21.9°C from the month of January to March and the relative humidity of the atmosphere was also increased from 50.5% in January to 53.5% in the month of March.

A total mean of 82.25 and 92.5 colonies were trapped from traffic circles on exposed Petri-plates at site I and Site II respectively after the sampling, which is shown in table II. A total mean of 60.75 and 70.25 colonies were trapped from residential areas on exposed Petri plates at Site III and Site IV respectively, which is shown in Table III. Maximum number of colonies were recorded in the later study period due to increase in temperature up to 23°C. The maximum numbers of colonies were counted from the exposed Petri plates (92.5c/m³) at site II, 82.25 c/m³ at site I followed by 70.25 c/m³ at site IV. The lowest numbers of colonies were recorded from Site III (60.75 C/m³). Table IV is indicating the relative occurrence of fungi at four different sampling sites.

During the course of study, the genus *Aspergillus* accounted maximum at site I (23.40%) and minimum at site IV (20.50%). Different species of *Aspergillus* were identified, including *Aspergillus flavus*, *Aspergillus fumigates* and *Aspergillus niger*. *Aspergillus flavus* was found maximum at site II (10%) due to regular human activities and heavy load of vehicular pollution. *Alternaria* was found in frequency on exposed plates at site II and site III accounting 7.02 % and 7.85% respectively of the total occurrence. *Alternaria* is a genus of ascomycete fungi. The occurrence of *Cladosporium* was found to be maximum 14.02% at site IV and minimum 9.72% at site I along with 10.27% at site II and 13.22 % at site III. *Cladosporium* is a genus of fungi including some of the most common indoor and outdoor molds. The percentage occurrence of *Fusarium* was found to be maximum at site III (6.19%) and minimum at site II (1.35%) along with (3.33%) at site I and (3.23%) at site IV. *Fusarium* is a fungi of class Deuteromycetes. *Helminthosporium* was found in higher frequency on exposed plates of site III and site I accounting 7.43% and 6.07% respectively followed by 5.13% at site II and 5.39% at site IV. Colonies of *Mortierella* were counted highest from the exposed petriplates at site II (6.48%) and lowest at site III (3.30%) followed by 6.38% at site II and 5.03 % at site IV. In the present study, the occurrence of *Mortierella* was more in traffic areas than the residential areas. Occurrence of *Mucor* was found to be maximum 12.58% at site IV and minimum at site II 10.54% along with 12.51% at site I and 12.39% at site III. The present study showed the maximum occurrence of *Nigrospora* 5.03% at site IV and minimum 3.95% at site I along with 4.59% at site II. *Nigrospora* was not present at site III. The occurrence of *Nigrospora* was more at traffic areas when compared with residential areas. In the present study, the percentage

occurrence of *Penicillium* was found to be maximum at site II with 10.54 % and minimum at 4.31 % at site IV along with 8.81% for site I and 4.54 % for site III. *Penicillium* is a genus of Ascomycetes fungi of major importance in the natural environment as well as food and drug production. The present study showed the highest percentage of *Rhizopus* at site IV (12.43 %) and minimum at site I (9.42%) along with (10.27%) at site II and (10.74%) at site III. *Rhizopus* is a fungus comes in order of Zygomycetes and grow as saprophytes on a wide variety of organic substates like bread, jellies, syrups, leather etc. The percentage occurrence of *Trichoderma* was (4.13%) at site III along with (4.05%) at site II, (3.95%) at site IV and 3.64% at site I. *Trichoderma* is a genus of fungi that is present in all soils, where they are the most prevalent culturable fungi. The colonies of *Trichoderma* are generally white to green in colour. There were some species which could not be identified during the study period. The percentage occurrence of unidentified species at site I was (6.99%) along with (6.75%) at site II, (8.67%) at site III and (6.83%) at site IV.

The abundance of airborne fungal spores reveal specific variation pattern which can be correlated with changes in metrological

factors such as temperature and relative humidity. During the entire work, the lowest temperature was during January (11.9°C), the fungal colonies and species were also decreased. An increase in temperature and relative humidity resulted in high occurrence of mycoflora. Afzal *et al.*, (2004) observed that increased degree of temperature caused significant increase in species and colonies of fungi during the summer season. Tan *et al.*, (1992) reported that spore load is related with relative humidity. Ahmad (1960) discussed that due to temperature and relative humidity, spore's productivity is affected. In the present study, during the month of March, there was increase in percentage of fungal colonies during the later study period which were associated with relative humidity and temperature, while in February, there were low no. number of fungal colonies due to decrease in relative humidity and temperature. The important factor on which the colony count depends is the type of media used. In this work, Potato Dextrose Agar media was used for the growth of fungi. The results are not easily compared mainly because of the different sampling techniques that different authors used. According to Sabariego *et al.*, (2007), very high temperature and absence of rain are unfavorable conditions for the growth of fungus.

Table.1 Values of atmospheric mean temperature and mean relative humidity during the study period

Month	Mean temperature (°C)± S.E	Mean relative humidity (%)±S.E
January	11.9±0.02	50.5±0.33
February	19.4±0.25	50.5±0.22
March	21.9±0.25	53.5±0.33

Table.2 Values of colony forming unit of fungal colonies after plate exposure at different traffic sites. (All values are mean± S.E. for 6 observation each)

Sl. No.	Site I (Singh Dwar)	Site II (Jatwara Pul)
I.	11.75 ± 0.85	13.75 ± 0.92
II.	14.25 ± 0.94	12.0 ± 0.86
III.	12.75 ± 0.89	14.25 ± 0.94
IV.	13.25 ± 0.91	16.75 ± 1.02
V.	13.0 ± 0.90	17.0 ± 1.03
VI.	17.25 ± 1.03	18.75 ± 1.08
Total CFU c/m ³	82.25 c/m ³	92.5 c/m ³

Table.3 Values of colony forming unit of fungal colonies after plate exposure at different residential sites. (All values are mean± S.E. for 6 observation each)

Sl. No.	Site III (Vishnu Garden Colony)	Site IV (Gyan Lok Colony)
I.	8.25 ± 0.72	12.0 ± 0.86
II.	9.5 ± 0.77	10.75 ± 0.81
III.	9.25 ± 0.72	11.5 ± 0.84
IV.	11.25 ± 0.83	11.0 ± 0.82
V.	10.0 ± 0.79	12.25 ± 0.87
VI.	12.5 ± 0.88	12.75 ± 0.89
Total CFU c/m ³	60.75 c/m ³	70.25 c/m ³

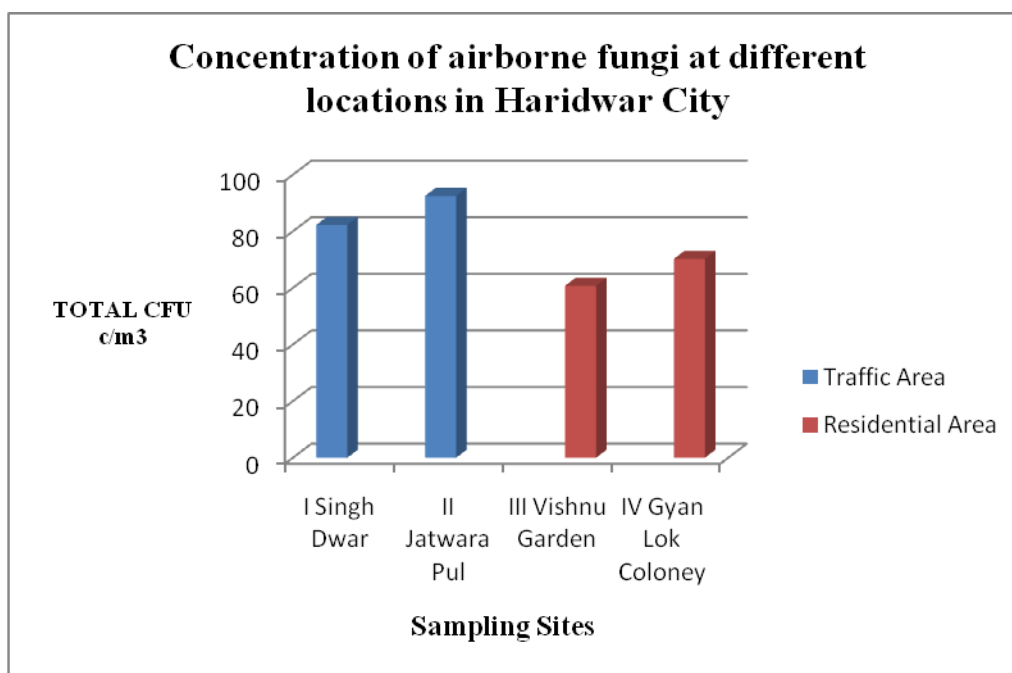


Figure.1 Values of colony forming units of Fungi at Traffic and Residential sites

Table.4 Relative Occurrence of Fungal Spores trapped through Cultured Petri plates at Different Sites

SL.No.	Name of Fungi	Traffic Area		Residential Area	
		Site I	Site II	Site III	Site IV
		R.O. of Species (%)	R.O. of Species (%)	R.O. of Species (%)	R.O. of Species (%)
1	<i>Aspergillus sp.</i>	23.40	22.43	21.48	20.50
I	<i>A.flavus</i>	7.90	10	6.19	5.03
II	<i>A.fumigatus</i>	6.38	5.13	7.02	8.99
III	<i>A.niger</i>	9.11	7.29	8.26	9.71
2	<i>Alternaria</i>	6.07	7.02	7.85	6.83
3	<i>Cladosporium</i>	9.72	10.27	13.22	14.02
4	<i>Fusarium</i>	3.33	1.35	6.19	3.23
5	<i>Helminthosporium</i>	6.07	5.13	7.43	5.39
6	<i>Mortierella</i>	6.38	6.48	3.30	5.03
7	<i>Mucor</i>	12.15	10.54	12.39	12.58
8	<i>Nigrospora</i>	3.95	4.59	—	5.03
9	<i>Penicillium</i>	8.81	10.54	4.54	4.31
10	<i>Rhizopus</i>	9.42	10.27	10.74	12.43
11	<i>Trichoderma</i>	3.64	4.05	4.13	3.95
12	<i>Unidentified sp.</i>	6.99	6.75	8.67	6.83

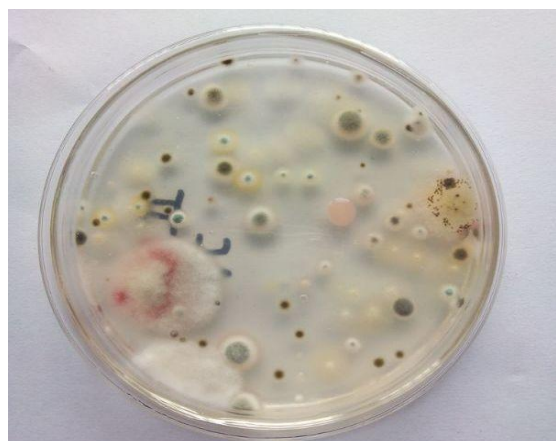


Photo plate 1
Showing early study of Fungal colonies at site I

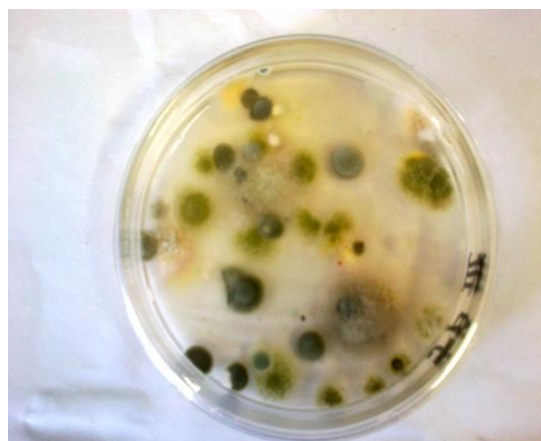


Photo plate 2
Showing later study of Fungal colonies at site I.



Photo plate 3
Showing early study of
Fungal colonies at site II.

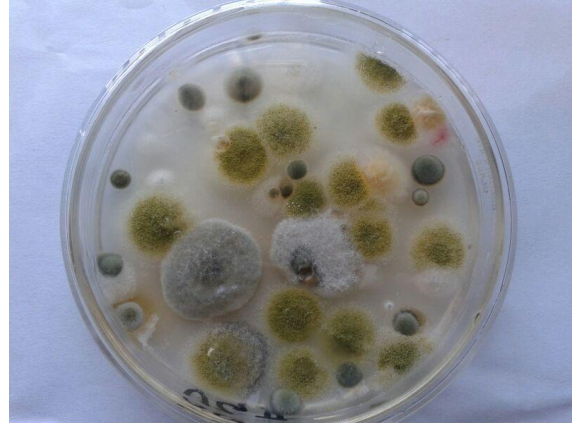


Photo plate 4
Showing later study of
Fungal colonies at site II.

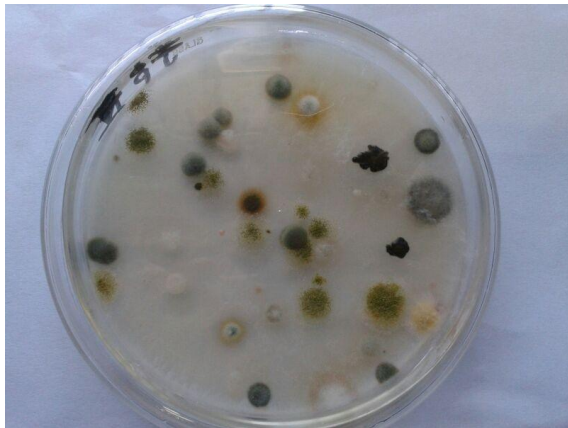


Photo plate 5
Showing early study of
Fungal colonies at site III.



Photo plate 6
Showing later study of
Fungal colonies at site III.

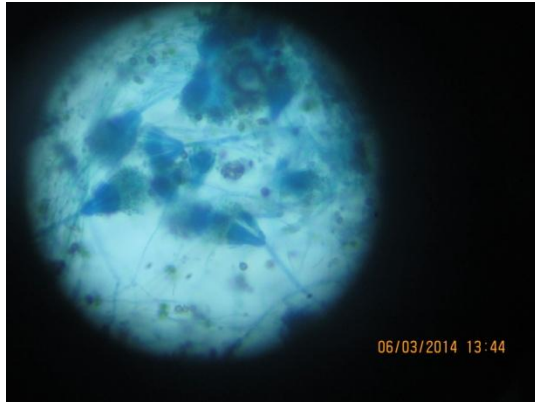


Photo plate 7
Showing early study of
Fungal colonies at site IV.

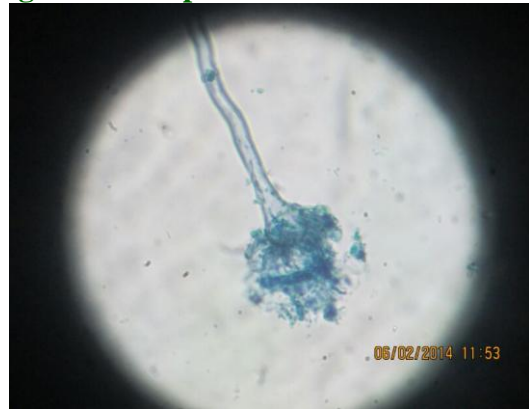


Photo plate 8
Showing later study of
Fungal colonies at site IV.

Some species which were recorded through microscopic examination at 45x



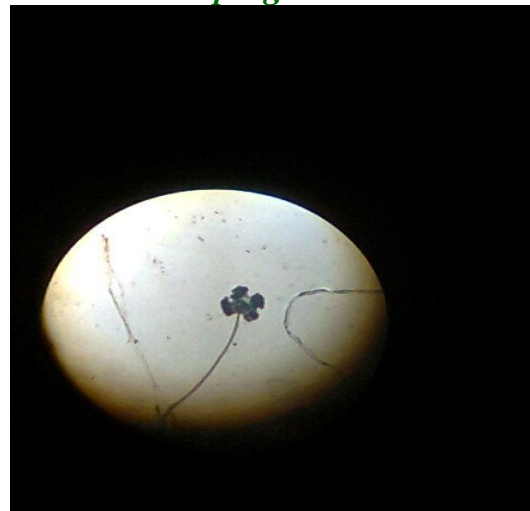
Penicillium



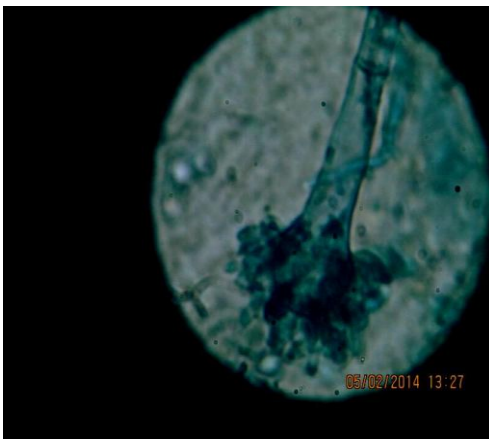
Aspergillus



Mucor



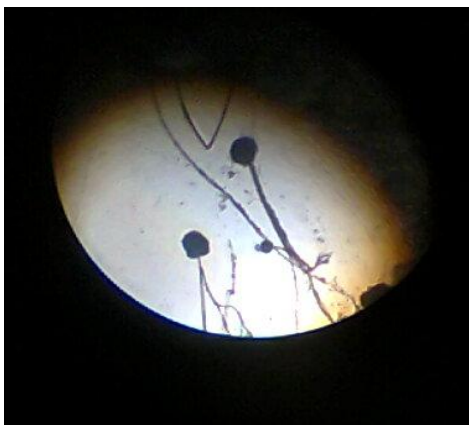
Aspergillus niger



Aspergillus fumigatus

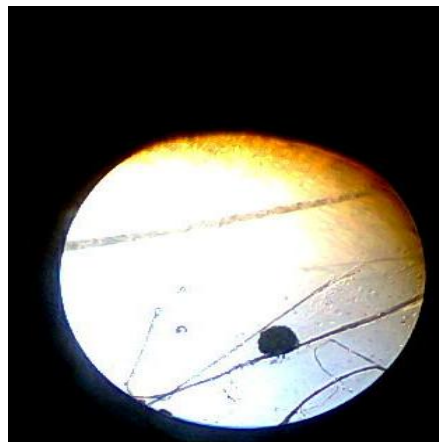


Mucor



Mortierella

In conclusion, the study revealed that the fungal spores were the major components of the air micro flora of both the outdoor and indoor environment of Haridwar city. The present study showed the variation related with the atmospheric temperature and relative humidity. The maximum number of colonies were recorded from both the outdoor and indoor environment in the month of March due to increase in temperature which showed that an increase in temperature and relative humidity of the atmosphere resulted in increased number of mycoflora qualitatively and quantitatively. (Afzal *et al.*, 2004). Site II has more number of colonies as compared to Site I, Site III, and Site IV due to the heavy vehicular pollution through buses, auto, etc. throughout the day and night and the presence of edibles and other discarded materials at site II.. Among the fungal spores caught from the air, *Aspergillus*, *Cladosporium*, *Mucor*, and *Rhizopus* were the dominant species at both the circles, traffic circles as well as residential circles. However, *Aspergillus* was reported as the most dominant species of the outdoor environment and *Cladosporium* and *Rhizopus* were reported as the dominant fungi at residential surfaces. Despite limitations of the technique employed and short duration of survey occurred in the area



Mucor microsporus

and has revealed the extent and nature of bio pollution of air due to fungal origin at public places. In the future, studies on the dependence of indoor fungal and microbial flora on socioeconomic and hygienic factors will be of interest. Such studies should clarify the relationship between indoor and outdoor fungal microbial flora.

Acknowledgement

I greatly express my profound gratitude to my guide, Prof. Namita Joshi, Department of Environmental Sciences, Kanya Gurukul Campus, Haridwar for constant support and advice and Dr. Sangeeta Vidyalankar, Co-ordinator, KGC, Haridwar for providing all necessary facilities to carry out the study.

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How to cite this article:

Raushani Raj and Namita Joshi. 2016. A Comparative Study of Aeromycoflora in Traffic and Residential Areas of Haridwar City, India. *Int.J.Curr.Microbiol.App.Sci.* 5(6): 161-170.
doi: <http://dx.doi.org/10.20546/ijcmas.2016.506.020>