

A report on *Penicillium* in the intramural and extramural air of residential areas of Nagpur city (India)

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Abstract

Prevalence of different species of *Penicillium* and their concentrations per cubic meter of air were evaluated with the use of Hi-Air sampler system Mark II (Hi-Media Laboratories Ltd., India) in the air of homes (bed-rooms) at four different sites in Nagpur. At each of these sites, air sampling was done fortnightly in triplicate for 2 years duration from June 2000 to May 2002. The sampling was also done in triplicate for the outdoor air in the vicinity of each home on the same day immediately after the indoor sampling was over. The mean concentration of *Penicillium* colony forming units at four different sites in the indoor air was 32, 46.9, 35 and 35.4 CFU/m³, respectively, whereas in the outdoor air at these same four sites, the mean concentration was 24, 28, 25 and 25.8 CFU/m³ respectively. The *Penicillium* concentration in the indoor air was found to be higher in winter than in other seasons (ANOVA, $p < 0.05$). Concentration of *Penicillium* spp. in intramural environment was always higher than that in extramural environment. Statistically significant difference existed between intramural and extramural environments at all the sites, with maximum difference at a site, which is old crowded area of the city. During the 2-year's investigations, 11 species of *Penicillium* were isolated from the indoor air while nine species were isolated from the air outside the homes. The dominant species of *Penicillium* in indoor as well as outdoor air were *P. citrinum* (33.78 and 32.81%), *P. oxalicum* (19.70 and 22.60%), and *P. chrysogenum* (17.64 and 14.50%). The percentage of the *Penicillium* in the indoor air was 10.70 while it was 8.36 in outdoor air. Indoor air showed the presence of *P. glaber* and *P. sclerotiorum*, which were absent in the outdoor air.

1. Introduction

The fungal presence in the air of different environments is being studied throughout the world by different methods like spore trapping, simple culture plate exposure method and the volumetric air sampling of viable and culturable spores. Keeping in mind the importance of allergenicity of the *Penicillium*, the study of this genus becomes essential in the different indoor and outdoor environments. The homes, and particularly the bed rooms, constitute the important environment for those individuals who spend most of their time in homes and are exposed to the airborne fungi in

this indoor environment. For the study of respiratory allergies, knowledge of airborne fungi indoors is perhaps even more important than that concerning propagules outdoors. However, very few studies have focussed on comparison of fungal spores both indoors and outdoors at the same time and in the same area.

Recent studies have reported a high degree of allergenicity in fungi like *Penicillium*, *Aspergillus* and *Neurospora* (Solley and Hyatt, 1980). Thus, the data on air-borne fungal spores, particularly *Aspergillus* and *Penicillium*, in the air inside homes in different regions of India is an essential prerequisite in the diagnosis and treatment of human allergy.

Aspergillus and *Penicillium* cannot be recorded as separate genera by spore trapping, since their spores are similar in size, shape and colour and are indistinguishable from one another. Though plenty of data is available from various regions of India, it is mainly in the form of spore concentration and therefore true revelation of the occurrence of *Penicillium* in the air has always remained incomplete. Thus, instead of the simple spore studies, the volumetric study of culturable (viable) spores becomes necessary, especially for *Penicillium*. In the present survey, therefore, the volumetric study of culturable spores by Hi-Air sampler was undertaken for air inside as well as outside the homes of different localities of Nagpur (a city in Central India) to reveal the prevalence of different *Penicillium* species.

2. Materials and methods

Prevalence of different species of *Penicillium* and the total fungal counts in cubic meter of air were

evaluated with the use of Hi-Air sampler system Mark II (Hi-Media Laboratories Ltd., India) in the air of homes (Bedrooms) at four different sites in Nagpur.

Nagpur is situated practically at geographical centre of India (Latitude 21° 09' N, Longitude 79° 09' E), in fact the zero milestone of India is in this city. It is 304 m (Mean height) above the sea level. The climate of Nagpur follows a typical seasonal monsoon weather pattern. The average maximum temperature of Nagpur is 42 °C while average minimum temperature is 13 °C, average annual rainfall is 1205 mm, and average maximum relative humidity is 70%, while minimum is 20%.

The four residential sites Civil Lines, Mahal, Nandanwan and Indora were randomly selected to make a representative sample (Figure 1). Two homes from each of these sites were selected randomly. Air sampling was done in the indoor air fortnightly in triplicate for 2 years duration from June 2000 to May 2002. Immediately after the

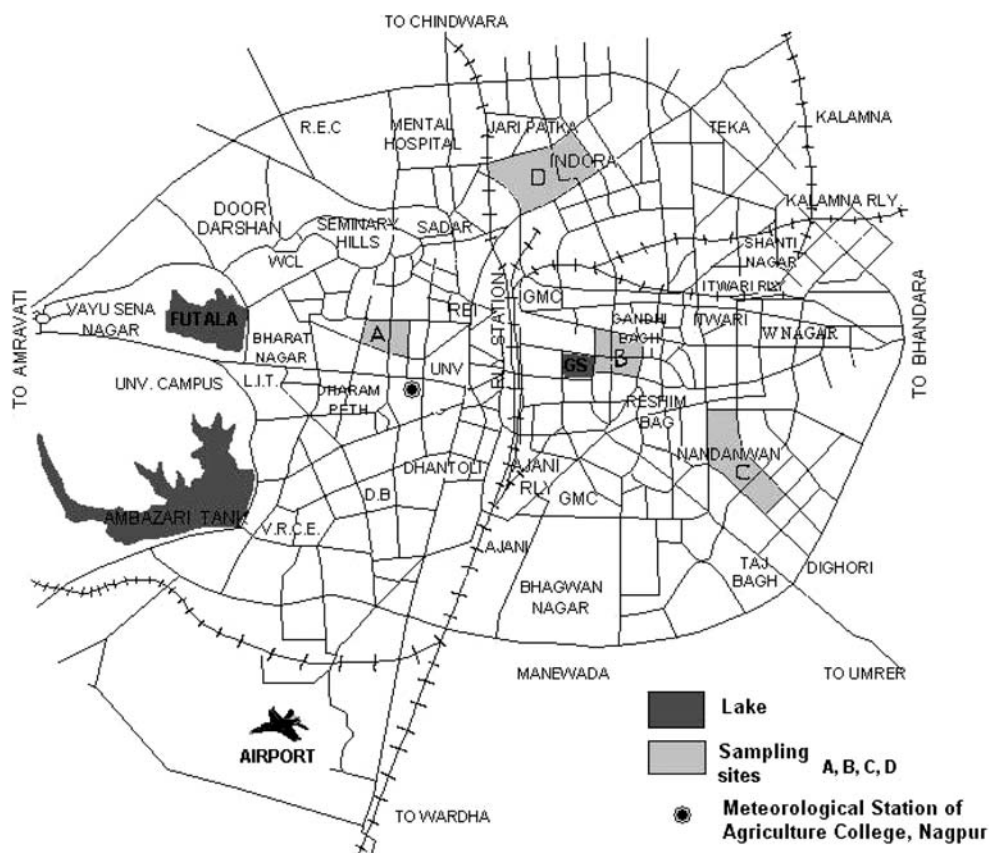


Figure 1. Map of Nagpur city showing the sampling sites.

Table 1. Showing mean total fungal concentration (CFU/m³) and *Penicillium* concentration (CFU/m³) in indoor and outdoor air at different sites and statistical significance of their difference at each site

Sites	Mean total fungi (CFU/m ³)		<i>t</i> Test, <i>p</i> value	Concentration of <i>Penicillium</i> (CFU/m ³)		<i>t</i> Test, <i>p</i> value
	Indoor	Outdoor		Indoor	Outdoor	
Civil lines	299.33 ± 16.6	258.63 ± 14.24	0.11	32.08 ± 3.46 (11.9%, 8)	24.1 ± 2.13 (10.3%, 7)	0.06
Mahal	399.16 ± 21.87	343.50 ± 18.2	0.049	46.92 ± 3.50 (12.6%, 9)	28.13 ± 2.15 (8.6%, 9)	0.00005
Nandanwan	347.50 ± 16.84	304.58 ± 16.06	0.07	35.0 ± 2.84 (10.6%, 8)	25.13 ± 2.04 (8.9%, 7)	0.0072
Indora	339.58 ± 19.13	299.29 ± 13.90	0.09	35.42 ± 2.69 (11.1%, 11)	25.83 ± 2.54 (9.4%, 8)	0.0128

The average values are depicted along with (±) standard error of mean.

Parentheses show the percentage contribution of *Penicillium* to total fungi and the number of *Penicillium* spp. isolated from that site.

indoor sampling, the outdoor sampling in the vicinity of the home was done.

Hi-Air sampler is a centrifugal impactor where air is impacted onto isolation medium contained in the strips. After the isolation of fungi by air sampler on agar strips containing Rose Bengal Streptomycin medium and incubation of strips at 27 ± 2 °C for 7 days, the total fungal count was expressed as colony forming units per cubic metre of air (CFUs/m³). *Penicillium* colonies were identified with the help of stereomicroscope and microscopic slides and then were sub-cultured on Czapek's Dox Agar medium and Malt extract Agar medium and identified at the species level with the help of published literature (Raper and Thom, 1949; Ramirez, 1982; Singh et al., 1991). The identification was then confirmed from Indian Type Culture Collection, Indian Agricultural Research Institute, Pusa, New Delhi.

The meteorological data was obtained from the Meteorological station of Agriculture College, Nagpur, situated in the heart of the city not more than 4 km from any of the selected sites.

Statistical analysis of the data obtained was done using appropriate statistical methods like ANOVA, Pearson's correlation coefficient, and 't' test with the help of statistical tools of Microsoft Excel.

3. Results and discussion

3.1. Total fungal concentration

In the residential areas of different localities of Nagpur, the total fungal concentration ranged from 150 to 690 CFU/m³ in indoor air while it ranged from 144 to 535 CFU/m³ in the outdoor

air. The mean values of total fungal concentrations at different sites are shown in Table 1. The month-wise total fungal concentrations in indoor and outdoor air are shown in Figure 2.

In Gwalior (India), Jain (2000) has reported the fungal concentration in different occupational indoor environments to be 37–431 CFU/m³. In a review article, Pasanen (1992) has reported that mean values of viable spore counts in residential environments in Europe and America ranged from 2 × 10¹ to 2.6 × 10³ CFU/m³ in 18 studies. The mean fungal concentration in the present investigation is comparable with these concentrations, though somewhat lower fungal catch in the present investigation may possibly be attributed to the use of a centrifugal impactor type of air sampler (Mehta et al., 1996). The fungal concentrations in the outdoor air in the present study are comparable to the findings of Amigot Lazaro et al. (2000) who reported 2.39 × 10² to 2.96 × 10² CFU/m³, Beaumont et al. (1985) who reported 2.6 × 10² CFU/m³, and Larsen (1981) who reported 2 × 10¹ to 1 × 10³ CFU/m³ in the outdoor air.

As the sampling methods, sampling periods and sampling sites differ, the results of the present investigation cannot be compared with those of many other researches (Rati et al., 1980; Bunnag et al., 1982; Bokhary and Parvez, 1995; Chakraborty et al., 2000; Kakde et al., 2001). In most of these studies relative concentrations, i.e. the number of colonies isolated during the experimental period and the relative percentages of different genera and species, have only been reported.

In the present investigation, at all the four sites, the mean fungal concentration was higher in the indoor air than in the outdoor air (Table 1). When the total fungal concentration in the indoor and outdoor air was compared with the help of 't' test,

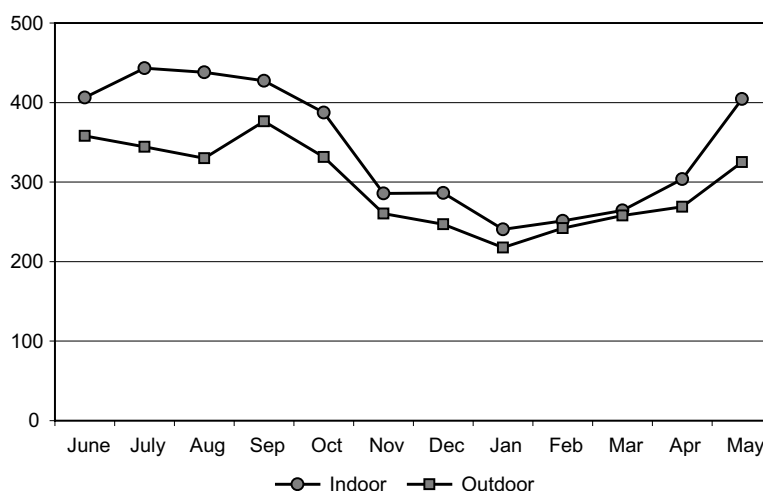


Figure 2. Monthwise concentration of total fungi (CFU/m³) in indoor and outdoor air.

statistically significant difference ($p < 0.05$) was observed only at Mahal area (Table 1), which is the densely populated area and the houses are old and poorly ventilated. The difference in the fungal concentration in indoor and outdoor air at this site can be attributed to the poor ventilation of the houses possibly resulting in increased humidity inside and to the wooden ceilings and crevices in the old walls and ceilings which act as the source of fungal amplification. In the homes which are well ventilated and open, outside particles can penetrate easily into the house and the fungal concentration become somewhat similar (Bunnag et al., 1982). Mycoflora of an indoor environment can depend both on the fungal spora coming from outside and the capacity of the fungi to colonise the different sub-layers found indoors as well as the differences in the environmental factors like temperature and humidity in different indoor environments.

In the present investigation, the difference in fungal concentration in indoor and outdoor air was maximum in the rainy season at all the four sites (Table 2, Figure 3). In the rainy season, outdoor fungal concentration increases to a lesser extent because of intermittent actual rainfall having cleaning effect on the aerospora while indoor concentration increases to a great extent due to high relative humidity and suitable substrates in indoor air.

The mean fungal concentrations in different seasons varied significantly (ANOVA single factor, $p < 0.01$), maximum concentration being in rainy season and lowest being in winter season for both

indoor and outdoor air (Table 2, Figure 3). Very good direct correlation was observed between relative humidity and minimum temperature suggesting low total fungal concentration in winter months (Table 3). Aidoo et al. (1995) also observed that the indoor aerial fungal spore counts were 51% higher in all the areas sampled during the warmer months than the colder months but there was no major change in the types of fungi. However, Portnoy et al. (2001) opine that climatic factors are only weakly associated with fungal contamination for the indoor air.

Goodman et al. (1966) observed that homes with evaporative cooling consistently showed a higher fungus count than was found in refrigerated homes. In the present study, somewhat higher fungal concentrations were observed in April and May in indoor air than outdoor air as compared to the concentrations in February and March. This fact may be explained by an evaporative cooling system present in almost all the homes in later half of April and May increasing humidity and fungal concentration inside (Figure 2).

3.2. *Penicillium* concentration

The mean concentrations of *Penicillium* in CFU/m³ at Civil Lines, Mahal, Nandanwan and Indora in indoor as well as outdoor air are given in Table 1. The average *Penicillium* percentage contribution as well as number of *Penicillium* species isolated at these four sites in indoor and outdoor air are also shown in Table 1.

Table 2. Average total fungal concentration and *Penicillium* concentration (CFU/m³) in different seasons along with statistical significance

Season	Total fungi (CFU/m ³)		't' Test, <i>p</i> value	<i>Penicillium</i> (CFU/m ³)		't' Test, <i>p</i> value
	Indoor	Outdoor		Indoor	Outdoor	
Rainy (June–September)	428.75 ± 14.50	352.25 ± 12.839	0.0000132	35.156 ± 3.110	22.187 ± 2.157	0.008
Summer (February–May)	305.93 ± 13.22	288.156 ± 13.48	0.0937	31.718 ± 2.329	26.718 ± 1.784	0.3502
Winter (October–January)	300 ± 13.82	264.09 ± 12.474	0.0081	37.43 ± 2.80	28.281 ± 1.794	0.058
ANOVA 'F' ratio	27.263	12.397		3.1103	2.717261	
df = 381, 2 <i>p</i> value	<i>p</i> < 0.01	<i>p</i> < 0.01		<i>p</i> < 0.05	<i>p</i> > 0.05	

The concentration is shown along with (±) standard error of mean.

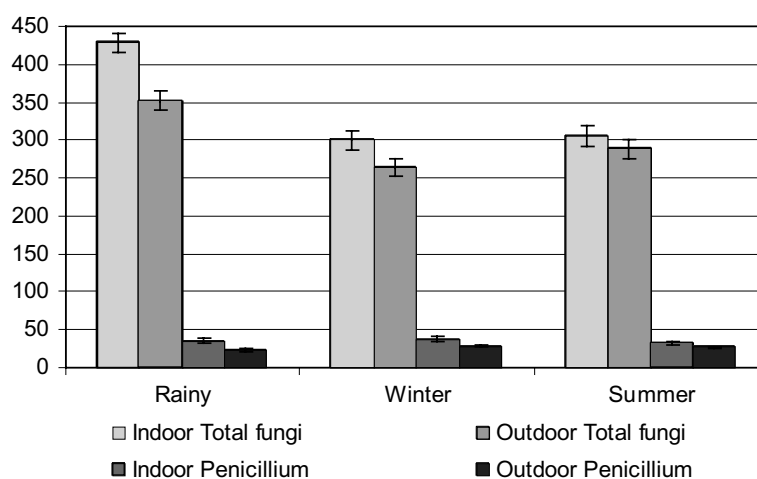


Figure 3. Seasonwise concentrations of total fungi and *Penicillium* (CFU/m³).

Table 3. Correlation coefficients (*r*) between different environmental parameters and total fungal concentration and *Penicillium* concentration in indoor and outdoor air

	Total fungal concentration		<i>Penicillium</i> percentage		<i>Penicillium</i> concentration	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Rainfall	0.56**	0.25	−0.34	−0.50**	0.00053	−0.44*
Relative humidity	0.56**	0.32	−0.06	−0.28	0.39*	−0.11
Minimum temperature	0.75***	0.76***	−0.68***	−0.77***	−0.23	−0.41*
Maximum temperature	0.18	0.40*	−0.43*	−0.29	−0.39*	−0.06

p* < 0.05, *p* < 0.01, ****p* < 0.001.

The dominant species of *Penicillium* obtained indoors were *P. citrinum* (33.78%), *P. oxalicum* (19.70%), *P. chrysogenum* (17.64%), *P. digitatum* (7.34) and *P. purpurogenum* (6.29%) while the dominant species isolated from outdoor air were

P. citrinum (32.81%), *P. oxalicum* (22.60%), *P. chrysogenum* (14.50%), *P. janthinellum* (8.51%) and *P. digitatum* (7.77%) (Table 4). Absolute *Penicillium* concentration was greater in winter than in rainy or summer seasons for indoor air

Table 4. Percentage contribution of different species of *Penicillium* to the concentration of total *Penicillium* spp. isolated from indoor and outdoor air

Species	Percentage in indoor air	Percentage in outdoor air
<i>P. chrysogenum</i>	17.64 (a, b, c, d)	14.50 (a, b, c, d)
<i>P. citrinum</i>	33.78 (a, b, c, d)	32.81 (a, b, c, d)
<i>P. digitatum</i>	7.34 (a, b, c, d)	7.77 (a, b, c, d)
<i>P. frequentans</i>	4.88 (a, b, c, d)	3.14 (a, b, c, d)
<i>P. glaber</i>	0.51 (d)	–
<i>P. italicum</i>	0.90 (b, c, d)	2.69 (b, d)
<i>P. janthinellum</i>	3.95 (a, c, d)	8.51 (a, b, c, d)
<i>P. minioluteum</i>	4.01 (a, b, d)	2.03 (b, d)
<i>P. oxalicum</i>	19.70 (a, b, c, d)	22.60 (a, b, c, d)
<i>P. purpurogenum</i>	6.29 (a, b, c, d)	5.95 (a, b, c)
<i>P. sclerotiorum</i>	1.02 (b, d)	–

Parentheses show the sites at which the species was present (a – Civil lines, b – Mahal, c – Nandanwan, d – Indora). Percentage contribution of all *Penicillium* species isolated to the total fungi was 10.70% in indoor air and 8.36% in outdoor air.

(ANOVA, $p < 0.05$; Table 2, Figure 3). The month-wise concentrations of *Penicillium* in indoor as well as outdoor air have been shown in Figure 4.

Many studies have reported *Penicillium* to be a dominant genus. Levetin and Hurewitz (1978) have reported *Penicillium* percentage varying from 5.0 to 41.7 in different homes with a mean of 21.1% in the air inside homes. *P. chrysogenum*, *P. citrinum*, and *P. oxalicum* were the dominant species in the study of Abdel Hafez et al. (1993). The dominance of these species was also observed in the present investigation (Table 4).

In the present study, *Penicillium* concentration correlated directly with relative humidity and inversely with the temperature (Table 3). *Penicillium* percentage in the air inside homes differed in different season and it was low in summer in a study of Levetin and Hurewitz (1978). The maximum isolation of *Penicillium* has been earlier reported in the months with low mean temperatures (Larsen and Gravesen, 1991; Abdel Hafez et al., 1993). This can be explained by the fact that *Penicillium* requires cooler climate and high humidity (Mehrotra, 1983), which is usually available in the winter season in Nagpur.

In the present study, *Penicillium* contributed 10.70% in the indoor air while its contribution to the total fungi in outdoor air was 8.36% though the composition of the genus was more or less same (Table 4). At all the sites except Civil Lines, statistically significant greater concentration of *Penicillium* was revealed in indoor air. At one of the sites (Mahal) which is the old part of the city, the difference in the *Penicillium* concentration indoors and outdoors was statistically very highly significant ($p = 0.00005$, Table 1). This may be attributed to the densely arranged houses with poor ventilation in the old crowded area increasing the humidity and favouring the growth of *Penicillium* indoors. The higher contribution of the *Penicillium* in the old part of the city has also been revealed by Al-Suwaine et al. (1999) in Saudi Arabia.

The greater concentration of *Penicillium* in indoor air than outdoor air has been reported by many researchers (Beaumont et al., 1984; Lehtonen et al., 1993; Li and Kendrick, 1995). These

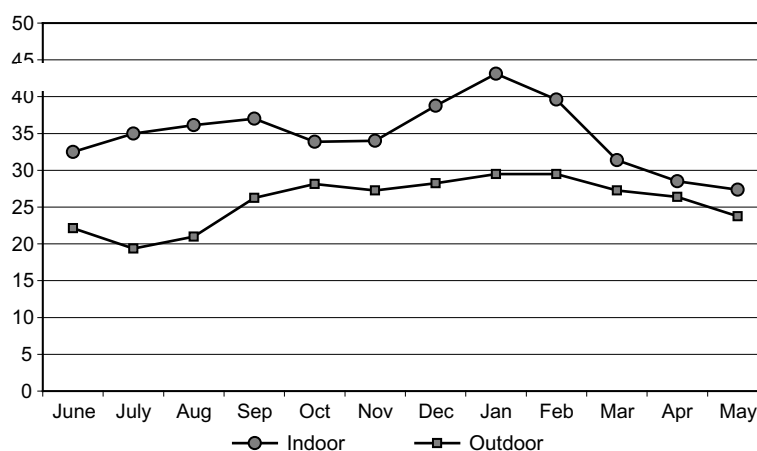


Figure 4. Monthwise *Penicillium* concentration (CFU/m³) in indoor and outdoor air.

workers have attributed the reason for this difference in indoor and outdoor air to the dispersal of conidia from the indoor sources and accumulation. The implication of the higher concentration of *Penicillium* indoors is that the spores of *Penicillium* growing indoors can cause allergic symptoms in mould sensitive subjects because they spend most of their times in homes (Tilak and Patil, 1981; Bunnag et al., 1982).

Penicillium spores are important aeroallergens. The high concentration of *Penicillium* spores are hazardous and they are known to cause respiratory tract disorders. Some of the species of this genus are mycotoxic while some species are considered to be true or opportunistic pathogens (Rati et al., 1980). Cooley et al. (1998) found that there were significantly more CFU/m³ of *Penicillium* in the air samples from rooms with complaint of sick building syndrome when compared with air of other area and outdoor air, suggesting a direct role of *Penicillium* in the sick building syndrome. The higher concentrations of airborne *Aspergillus*/*Penicillium* species in the homes have been linked to the respiratory ailments like allergy and asthma (Agashe et al., 1994; Portnoy et al., 2001). The consistent presence of *Penicillium* with more concentration in indoor air than that of outdoors and obvious rise in concentration in winter months suggests a possible role of *Penicillium* in causing allergy in this region, which is often neglected because of dominance of and vast studies on *Aspergillus*.

4. Conclusion

In this survey, *Penicillium* spores from different species were found to be present in the air of both outdoor and indoor environments throughout the year with greater concentrations in the indoor environment particularly during winter months. The exacerbations of the allergenic respiratory disorders may have a possible correlation with higher *Penicillium* concentrations of some allergenic *Penicillium* species in this region. This also suggests a need to study at least the dominant *Penicillium* species for its allergenic potential. In our future work, the studies involving allergenicity testing of different dominant species of the genus *Penicillium* in this region will be undertaken.

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