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A preliminary investigation on airborne fungi of pedestrian underpasses

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Abstract Pedestrian underpasses are the unique environment where air transaction with outdoor is complicated, inducing interest to study the air quality for the presence of microbes. Thus, the study on the presence of airborne fungi from 11 different underpasses in Chennai city was conducted. The airborne fungi was monitored by exposing potato dextrose agar strips in reuter centrifugal sampler (RCS). A total of 1735 colonies were isolated from underpasses and the control site recorded only 1255 colonies. Total average colony forming unit of underpasses and control site was 157.7 CFU/m^3 of air and 114.09 CFU/m^3 of air, respectively. Altogether 22 species classified in 16 genera were isolated from both the environments. Of which 21 species belonging to 16 genera were isolated from underpasses whereas the control site yielded 15 species belonging to 12 genera. The isolated species were categorized into three species belonging to Zygomycota, 2 species to Ascomycota and the remaining to Mitosporic fungi. The air sample in the underpass is dominated by the presence of the fungi, *Aspergillus flavus, Curvularia lunata* and *A.niger*, whereas the control is dominated by *Cladosporium cladosporioides, Aspergillus niger* and *A. flavus.* The study proves the underpasses are a favorite niche for the proliferation of fungal propagules and proper maintenance of underpass is recommended.

Keywords Airborne fungi · Underpasses · Chennai · RCS · Average CFU/m^3 · *Aspergillus*

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1 Introduction

Cities in general are characterized by rapid urbanization, development of industries, highly populated and possess heavy traffic. In addition, explosion of population due to the movement of people from rural area to urban cities, the infrastructure needs to be improved. The increase in population results in the movement of people for their daily business and economic activities and thus increase in traffic. The economically weaker section use to walk as this is the simplest mode of transport. But, they are the most vulnerable group to road accidents (Pasha et al. 2015). The pedestrians need to face variety of road traffic and intensity of traffic mix. Traffic on road leads to

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premature road accidents which effects the socioeconomic state of the country. Nearly 85% of injuries and death in low and middle-income countries are reported. In India, 78% of road fatalities were pedestrians (Mohan 2002). Thus to prevent road accidents, the city corporation develops underpasses at various traffic junctions for the safe movement of pedestrians. The Greater Chennai Corporation is one of the metropolitan city of India and is the capital of the state of Tamil Nadu. The city has a spread over area of 426 sq. kilometers which is well connected by all means of transportation including air, water and land. The road network of Chennai city is 2780 km. The city is an economical hub which provides livelihood for nearly 8.7 million people. There are many underpasses constructed in city junction among which 11 major underpasses were studied for their presence of air mycoflora.

Pedestrian underpasses in Chennai also possess commercial activities making it as a business center with petty shops. This also provides shelter to the destitutes. Pedestrian underpass is a microclimatic environment which lacks natural light and are illuminated with street lights. Most pedestrian underpasses are badly maintained with dampness and leakage during rainy season. The temperature, moisture content and humidity are high in underpasses. It is a known fact that the air current in underpasses is quite different than that of outdoor as the airflow within underpass is condensed due to the negative pressure. Thus, the air quality of these microclimatic environment is under question. It is important to know the presence of microbes, especially the fungi a major air component which pose various health effects as allergic, toxic and pathogenic to pedestrian underpass users. There are many studies on airborne fungi of rail subways (Hoseini et al. 2013; Hwang and Cho 2016; Xu and Hao 2017), rail station (Bogomolova and Kirtsideli 2009), mining tunnels (Gebarowska et al. 2018) and utility tunnel (Wu et al. 2019). However, the literature search on the study of airborne fungi of pedestrian underpasses revealed that there was no study conducted so far. This study is first of its kind reporting the presence of airborne fungi from 11 different pedestrian underpasses of Chennai city, India.

2 Materials and methods

2.1 Sampling site

Chennai, one of the major metropolitan cities of India, is the capital of the state of Tamil Nadu. The city is situated at the latitude of 13.0827° N, longitude of 80.2707° E and altitude level of 6.7 m above sea level. The city has a huge population which is exceeding 8.7 million due to rapid urbanization and industrialization. A total of 11 pedestrian underpasses located in major road junctions of the city were monitored for airborne fungi. The location of pedestrian underpasses where air samples were collected in Chennai is represented in the city map (Fig. 1).

2.2 Air sampling

The air samples were collected using a volumetric sampler, reuter centrifugal sampler (RCS) (Biotest AG, *Landsteinerstraße* Dreieich, Germany). It is portable and operated using batteries. The agar strips containing sterile potato dextrose agar (PDA: Potato-200 g, Dextrose-20 g, Agar-20 g, distilled water-1000 mL) (Himedia Inc. Mumbai, India), supplemented with streptomycin, were used for the isolation of fungi. The air sampler was operated for 5 min with the suction rate of 40 L/min and at the height of 1 m from the ground. The exposed PDA strips were incubated at room temperature (28 ± 2 °C) for 4–5 days. The developing fungal colonies were counted and identified using macroscopic and microscopic features using standard manuals (Subramanian, (1971), Onions et al. (1981), Ellis (1971) and Udayaprakash (2004)).

2.3 Presentation of data

The average colony forming units of fungi isolated per cubic meter (CFU/m^3) of air, their percent contribution and isolation frequency were calculated as follows:

2.4 Colony forming units (CFU/)/cubic meter of air (m^3)

2.4.1 Conversion factor:

The colonies of individual species are converted to number/m^3 of air by multiplying with a factor calculated as follows and the counts are expressed as.

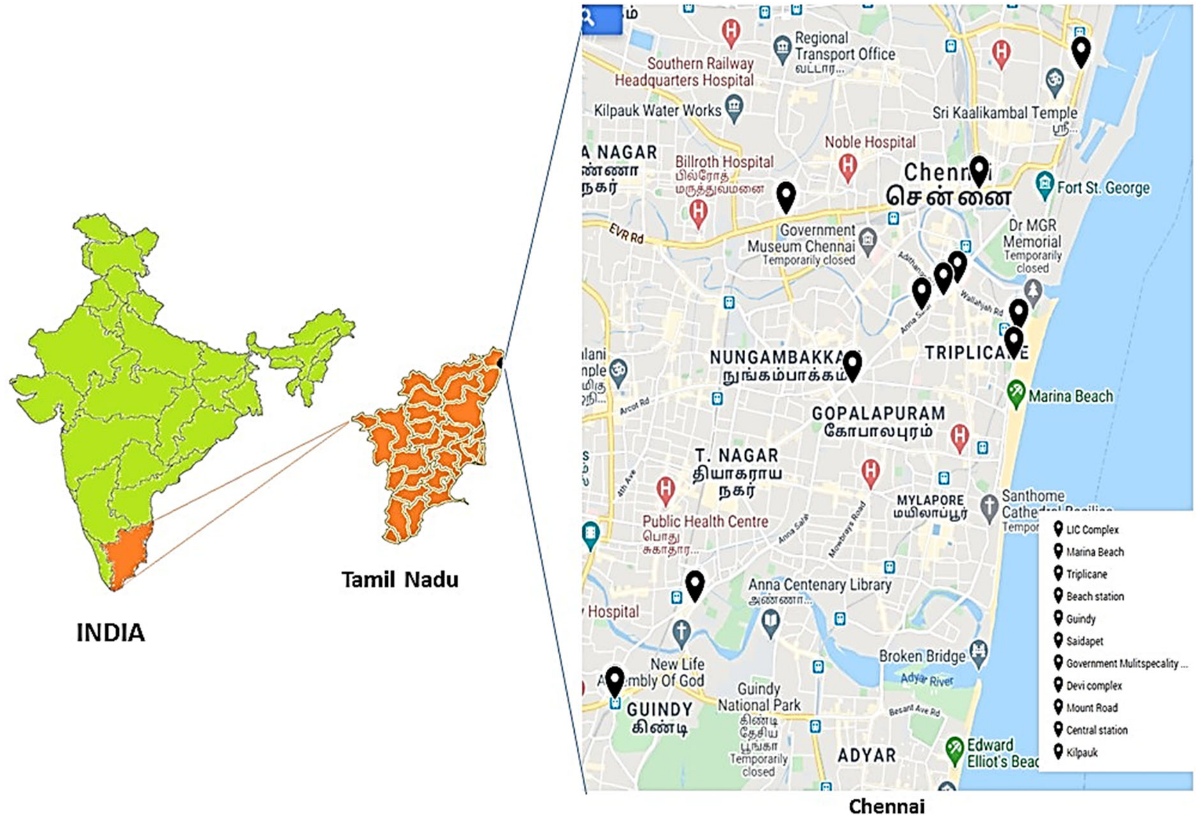

Fig. 1 Chennai city map showing the sampling site of different underpasses

Suction rate of the sample = 40.

Duration of each sampling = 5 min.

Amount of air sampled in 5 min = 40 × 5 = 200.

Let the number of colonies recorded = X

$$\text{Colony formingunits}/m^3 \text{of air} = \frac{200}{1000}(X)$$

$$\text{Percent contribution} = \frac{\text{Total CFU}/m^3 \text{of an individual species}}{\text{Total CFU}/m^3 \text{ of all species}} \times 100$$

$$\text{Percent occurrence} = \frac{\text{No. of samples in which a species occurred}}{\text{Total no. of samples}} \times 100$$

3 Statistical analysis

The biodiversity indices, viz., Shannon-Weiner index (H), Gleason index, Simpson reciprocal index, Simpson dominance index (D), Pielou evenness index (J), Jaccard index (J(X,Y)) and were calculated according to the following formulae:

Shanon-Weiner index: $H = \sum_{i=1}^{S} -(P_i * \ln P_i)$.

Gleason index [H_G]: $N_{P-1}/\ln\ N_i$.

Simpson reciprocal index: $SR = 1/\sum_{i=1}^{S} Pi^2$.

Simpson dominance index: $\mathrm{SD} = \sum_{i=1}^{S} Pi^2$.

Pielou evenness index: $J = \frac{H}{\ln S}$.

Jaccard index: $J(X,Y) = \frac{X \cup Y}{X \cap Y}$where

H = the Shannon diversity index.

S = numbers of species encountered.

P_i = fraction of the entire population made up of species i.

Ni = total number of fungal isolates.

Np = total number of species to which these fungi belong.

$X \cup Y$ = number of similar species in both the sites.

$X \cap Y$& N = total number of species in the sample.

4 Results

A total of 1735 fungal colonies were isolated within pedestrian underpass and 1255 colonies were isolated in control site. The isolated colonies were classified into 22 species belonging to 16 genera apart from nonsporulating colonies. Among the isolated species, three species belong to Zygomycota, 2 to Ascomycota, and the remaining 17 belongs to Mitosporic fungi. The only genus representing more number of species is genus *Aspergillus* with 7 species and all other genera were represented by single species each. The mitosporic fungi contributed around 81.81% to the total. The average cfu/m^3 of air recorded within pedestrian underpasses ranges from 125 to 245 CFU/m^3 and in outdoor it was 45 to 285 CFU/m^3 of air (Fig. 2). A total average of 157.73 CFU/m^3 was recorded within pedestrian underpasses where as in control site it was 114.09 CFU/m^3.

The dominant fungi recorded from pedestrian underpasses was *Aspergillus flavus* with an average of 34.54 CFU/m^3 of air followed by *Curvularia lunata* with an average of 29.09 CFU/m^3 and *Aspergillus niger* with an average of 24.09 CFU/m^3 of air. In outdoor, it was *Cladosporium cladosporioides* which dominated with an average of 22.72 CFU/m^3 of air followed by *A. niger* and *A. flavus* with an average of 20.45 CFU/m^3 and 17.27 CFU/m^3 of air, respectively. The fungus, *Aspergillus flavus* alone accounted for 21.90% to the total, followed by *Curvularia lunata* (18.44%) and *Aspergillus niger* (15.27%) in underpasses. In control site, *Cladosporium cladosporioides* contributed around 19.92% to the total followed by *Aspergillus niger* (17.92%) and *A. flavus* (15.13%). The list of fungal species isolated from pedestrian underpass and control site, their average CFU/m^3 of air, percent contribution of individual species to the total and percent occurrence is presented in Table 1.

The prevalence of fungal species is concerned, *Aspergillus flavus, A. niger* and *Curvularia lunata* were isolated from 100% of air samples in pedestrian underpasses. The other prevalent fungi recorded from the environment includes *A. tamarii* which occurred in 72.73% of air samplings and *Syncephalastrum racemosum* and *Aspergillus japonicus* occurred nearly in 45% of the samplings studied. In control site, the

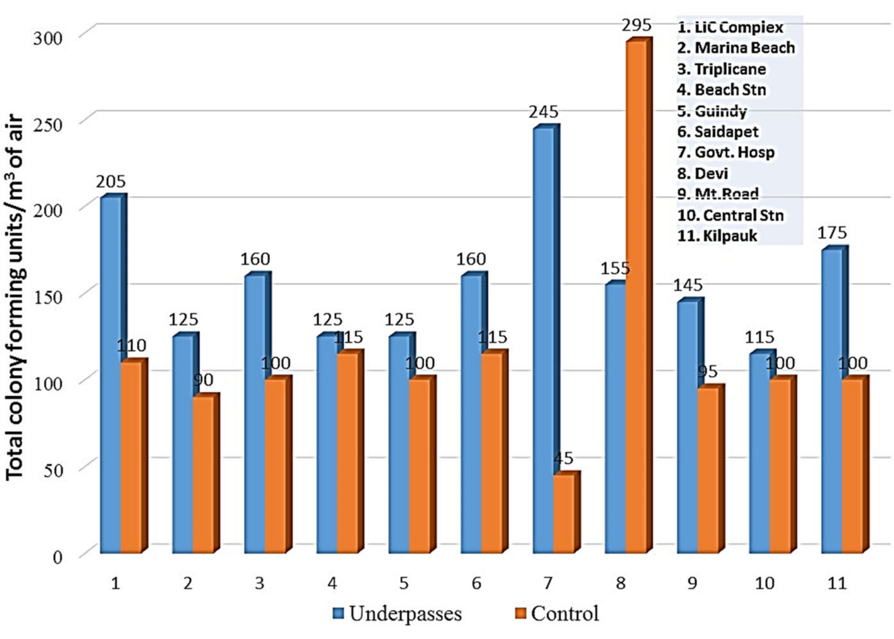

Fig. 2 Total CFU/m^3 of air recorded for different underpasses in Chennai city

Table 1 List of fungal species isolated from underpasses and control, their average CFU/m^3, percent contribution and Percent occurrence

Sl.No	Species	Average CFU/m^3 of air	%C	%O	Average CFU/m^3 of air	%C	%O
	Zygomycota						
1	*Absidia corymbifera*	1.36	0.86	9.09	0	0	0
2	*Rhizopus stolonifer*	1.36	0.86	9.09	3.63	3.18	9.09
3	*Syncephalastrum racemosum*	8.63	5.47	45.45	4.54	3.98	27.27
	Ascomycota						
4	*Chaetomium globosum*	1.36	0.86	18.18	0	0	0
5	*Emericella nidulans*	0.90	0.57	18.18	0.45	0.39	9.09
	Mitosporic Fungi						
6	*Alternaria alternata*	0.90	0.57	9.09	1.36	1.19	18.18
7	*Aspergilllus flavus*	34.54	21.90	100	17.27	15.13	63.63
8	*A. glaucus*	3.18	2.01	9.09	0	0	0
9	*A. japonicus*	5	3.17	45.45	5.45	4.78	45.45
10	*A. niger*	24.09	15.27	100	20.45	17.92	81.81
11	*A. ochraceus*	0.90	0.57	18.18	0	0	0
12	*A. tamarii*	12.72	8.06	72.72	5.90	5.17	45.45
13	*A. ustus*	1.36	0.86	9.09	0	0	0
14	*Aureobasidium pullulans*	0.45	0.28	9.09	0	0	0
15	*Chrysonilia sitophila*	14.09	8.93	27.27	4.54	3.98	9.09
16	*Cladosporium cladosporioides*	0	0	0	22.72	19.92	18.18
17	*Curvularia lunata*	29.09	18.44	100	15	13.14	72.72
18	*Drechslera australiensis*	2.27	1.44	27.27	1.36	1.19	9.09
19	*Fusarium oxysporum*	2.72	1.72	9.09	0.45	0.39	9.09
20	*Paecilomyces variotii*	0.90	0.57	18.18	0	0	0
21	*Penicillium oxalicum*	1.36	0.86	9.09	2.27	1.99	18.18
22	*Trichoderma viride*	0.90	0.57	9.09	2.27	1.99	9.09
	Non sporulating	9.54	6.05	81.81	6.36	5.57	45.45

% C = Percent Contribution

% O = Percent occurrence

dominant fungus, *Cladosporium cladosporioides* occurred only in 18% of the samples. The other dominant fungi, i.e., *A.niger* was recorded in 81.81% and *A. flavus* in 63.63% of the samples. Although the fungus, *Curvularia lunata* was not recorded as dominant, it occurred in 72.72% of air sampling. The other species, *Aspergillus japonicus* and *A. tamarii* were recorded in 45% of samplings studied. A total of 14 species were commonly isolated from both within underpasses and control site. However, the following 7 species, i.e., *Absidia corymbifera, Chaetomium globosum, Aspergillus glaucus, A. ochraceus, A.ustus, Aureobasidium pullulans* and *Paecilomyces variotii* were isolated exclusively within pedestrian underpasses and only one species *Cladosporium cladosporioides* was recorded from control site (Fig. 3).

The statistical analysis of the obtained data was evaluated for their richness, diversity, dominance and evenness of isolated species from the pedestrian underpasses and control. The Gleason index proves that species richness was high in the underpasses than control. The Simpson reciprocal index and Shanon Weiner index showed that the control site was species diverse. Less value of Simpson dominance index proves only few number of dominant species were isolated from both the sites. Similarly, low evenness

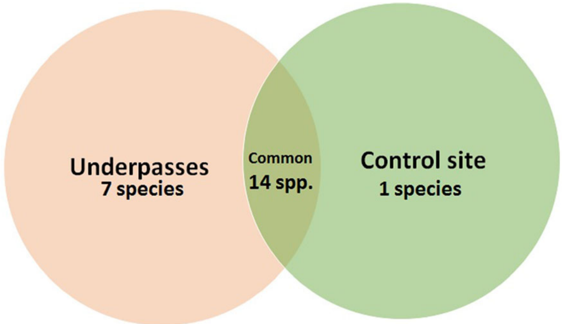

Fig. 3 Diagrammatic representation of species diversity recorded in underpasses and control site in Chennai, India

was recorded for both the site according to Pielou's evenness. The Jaccard index shows 63% of similarity exist between the species of underpasses and control. The statistical analysis of the data is presented in Table 2.

5 Discussion

The pedestrian underpasses is an infrastructure development by the government in major cities for the safe movement of people and to prevent road accidents. In underpasses, there are many petty shop and pavement shop keepers who spend nearly 10 h within underpass. The street dwellers also reside in these underpasses. The people prefer pedestrian underpass to cross the major roads rather than facing heavy traffic on roads. The underpasses are heavily congested during peak hours of the day. The air within underpasses are quite different when compared to control (Outdoor site). The outdoor air flushes through the entries develops increased atmospheric pressure within underpass resulting in increased temperature. The condensation of molecules in air results in high humidity and moisture. This unique microclimate of pedestrian underpasses is the hub of various microbes especially the fungi which is ubiquitous and one of the major component of air in the environment posing health risk to the underpass users. There are plenty of literature available on studies related to airborne fungi in rail subways of different cities in the world. The rail subway of Seoul, Korea (Cho et al. 2006); Tokyo, Japan (Kawasaki et al. 2010); Mexico city, Mexico (Castillo et al. 2014); and Beijing, China (Fan et al. 2017) were already reported. The studies on rail stations (Ghosh et al. 2011), tunnel mining (Rdzanek et al. 2018) and utility tunnel (Wu et al. 2019) were also reported. The above-mentioned studies were confined to the subways of metro rails or mass rapid transport systems (MRTs). However, study pertaining to airborne fungi of pedestrian underpasses is not available so far.

Table 2 Statistical analysis of the airborne mycoflora of underpasses in Chennai, India

Statistical Analysis	Underpasses	Control
Gleason index	3.954657	2.9559025
Simpson reciprocal index	7.934503	8.1983342
Shannon Weiner Index	0.0411	0.109752
Simpson dominance index	0.129716	0.1255
Pielou Evenness ratio	0.0135	0.041
Jaccard similarity	0.63636364	

Among the 22 species isolated, 14 species were commonly isolated from both the environments. This is due to interexchange of air between underpasses and the control sites. The present study shows an average of 157.7 CFU/m^3 of air in underpass which is higher in value than control site with an average of 114.09 CFU/m^3 of air. The increase in average CFU/M^3 of air in underpasses is attributed to the prevalence of microclimatic factors like temperature, humidity and atmospheric pressure which favors the proliferation of fungal spores within underpasses. Further, the human activities, i.e., disposal of waste, water and food material within underpass by the dwellers create a conducive environment for microbial growth.

The dominant fungi isolated from underpass and control was *Aspergillus flavus, A. niger, Curvularia lunata,* and *Cladosporium cladosporioides*. The size of fungal spores, which are lesser than 5 µm when inhaled has the ability to reach the alveolar region of the lungs. They have the potency to cause various respiratory symptoms like sneezing, running nose, allergic rhinitis, allergic alveolitis, breathlessness, asthma and in severe cases causes invasive Aspergillosis resulting in fatality. The presence of *Curvularia lunata* in underpass proves the availability of organic debris which acts as substrate for the growth of the fungi. The fungus is reported as cellulolytic and saprophytic fungus degrading the leaf litters. The percent occurrence of *Syncephalastrum racemosum* in 45% of air samples proves that most of the underpasses have high moisture content and dampness

(Nevalainen 2015). The control site is dominated by *Cladosporium cladosporioides* which is a common fungi reported in outdoor by many workers such as Mallo et al. (2011) and Shams-Ghahfarokhi (2014). From this study, it is proven that the knowledge on airborne fungi from underpasses is important and it relates to the health of underpass users. The present study is the first report on airborne fungi in pedestrian underpasses.

The proper maintenance of underpasses is recommended by adapting following measures, i.e., (1) cleaning the underpasses by sweeping twice a day (2) regular check by the officials for crack and damage (3) removal of dampness (4) spraying of disinfectants (5) installation of exhaust fans and (6) creating awareness to shop keepers and dwellers for proper maintenance.

6 Conclusion

The air samples of 11 different underpass and control site in Chennai city, India, were studied for the presence of airborne fungi. A total average of 157.7 CFU/m^3 of air was recorded from underpass and an average of 114.09 CFU/m^3 of air were recorded from control. A total of 22 species was isolated from both underpass and control site. The underpass is dominated by the presence of *Aspergillus flavus, Curvularia lunata* and *A.niger*, whereas the control is dominated by *Cladosporium cladosporioides, Aspergillus niger* and *A. flavus.* These fungi pose a risk of respiratory disorder to the users. The study recommends proper maintenance of underpass.

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Author contributions Conceptualization: SB, NKUP; Sample collection and preparation for analysis: IS, FF; Acquisition of data: IS, FF; Analysis and interpretation of data: SB, NKUP; Writing—original draft: SB, NKUP.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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