Eco. Env. & Cons. 28 (January Suppl. Issue) : 2022; pp. (S358-S364)
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ISSN 0971–765X

http://doi.org/10.53550/EEC.2022.v28i01s.050

Assessment of airborne fungi in the indoor environment of schools in Imphal

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(Received 20 August, 2021; Accepted 22 September, 2021)

ABSTRACT

Poor indoor air quality in schools has often been linked to increasing health impacts among school children. Airborne fungi may become one of the primary contaminants in the classrooms besides particulates, chemicals, and other pollutants. The current study aimed to assess the airborne fungi in the schools in Imphal city, Manipur. The investigation was carried out by using a two-stage Andersen air sampler. The fungi were isolated and identified based on morphological and growth characteristics. The highest indoor fungal concentration (587.75 CFU m^{-3}) was isolated in the school located at the heart of the city, and the lowest (465.96 CFU m^{-3}) was isolated in the school near the residential area. The concentrations of fungi in the outdoor air ranged between 543.62 and 645.99 CFU m^{-3}. The fungal concentration was highest during February and lowest during January. The fungal species isolated in the classroom and their respective contribution percentages to the total fungal concentration load were *Cladosporium* (38.24 %), *Aspergillus* (15.29 %), *Curvularia* (11.76 %), *Alternaria* (10.20%), *Penicillium* (9.08 %), *Fusarium* (8.04 %) and *Rhizopus* (2.75 %). The indoor-to-outdoor (I/O) ratios with less than one indicate exogenous sources of indoor fungal contamination. There was a significantly positive correlation (*r=0.99 at p<0.001*) between the indoor and outdoor fungal concentrations.

Keywords: *Indoor air quality, School children, Imphal city, Fungal contamination, Sick building syndrome*

Introduction

The airborne microbes in the form of suspended aerosols are present throughout the environment. The primary sources of these microbes are soil, water, plants, and animals. These microbes affect human health and reduce the quality of the environment (Ruzer and Harley, 2005). Certain environmental conditions support the growth and development of such microorganisms. In the right circumstances, microorganisms can thrive and proliferate in levels, causing health problems. Temperature, humidity, oxido-reductive potential, hydrogen ion concentration, water availability, and hydrostatic pressure in the environment are the main factors affecting the growth of microorganisms (Stanaszek-Tomal, 2020). Exposure to fungus causes acute toxic effects, allergies, and asthma (Bush and Portnoy, 2001). Fungal bioparticles may cause allergic rhinitis, asthma, allergic alveolitis, and bronchopulmonary aspergillosis (Kim *et al.*, 2007). More than 80 fungal genera are typically associated with respiratory allergies (Horner *et al.*, 1995). Fungal allergy affects nearly 10% of the world's population (Burge, 2001).

Fungi are common in the indoor and outdoor environment (Portnoy *et al.*, 2005; Baxi *et al.*, 2016). Their concentrations may be more, particularly in buildings often attended by many people (Meriggi *et al.*, 1996). The prevalence of fungi in the indoor air

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depends on the surrounding environmental conditions, primarily the microclimatic factors, fungal substrates, human activities, *etc.* Good indoor air quality (IAQ) is crucial for human well-being since modern men spend more of their time indoors (Dacarro *et al.,* 2003; Chen and Zhao, 2011). Airborne fungal spores and pollen grains are known to cause discomfort in occupational environments (O'Rourkey *et al.,* 1989; Lacey 1990; Dutkiewitcz *et al.,* 2001). A damp indoor environment increases the risk of fungal exposure, causing asthma in young children (Baxi *et al.,* 2016). Schools are the first place of social activity for young children and the next important indoor environment after home. Children spend almost 25-30% of their daily time inside classrooms (UNESCO, 2009). Children's developing bodies are more vulnerable to environmental exposures than adults as they breathe more air, consume more food, and drink more liquid than adults in proportion to their physical weight. Deterioration of indoor air quality in schools is increasingly recognized as a matter of great concern (WHO, 2010; USEPA, 2021). Singh and Gangal (2002) expressed that airborne bio-contaminants in the indoor air have become a significant health concern in India. The current study attempts to determine the status of IAQ with respect to the airborne fungal contamination in five schools in Imphal city of Manipur, India.

Materials and Methods

Study sites and schools

Five schools in Imphal city (24.721^0 N & 24.883^0 N and 93.887^0 E & 93.982^0 E) were selected for the investigation. The schools are (i) Johnstone Higher Secondary School (S-1); (ii) Lamlong Higher Secondary School (S-2); (iii) Ananda Singh Higher Secondary Academy (S-3); (iv) Churachand Higher Secondary School (S-4) and (v) Ibotonsana Girls Higher Secondary School (S-5). The classrooms in all the schools relied on natural ventilation and natural light.

Indoor and outdoor air sampling

Indoor (classroom) and outdoor ambient air samples were collected from the schools at monthly intervals from November 2018 to February 2019. Andersen two-stage sampler was used to collect samples (Andersen, 1958). The sampler was placed 1.5 meters above the ground level. Potato dextrose agar (PDA) was used as a sampling medium. The air is sucked into the sampler through a circular orifice and a series of 2 circular plates, each having 200 perforations through which air and bio-particles are impacted directly into the sterile medium in the Petri dishes fitted inside the orifice. During the sampling, airflow inside the sampler's inlet orifice was 28.3 L min^{-1}, and the air sampling time was carried out for 5 minutes during the school hours.

Fungal identification and counting

After the sampling, the air sampling plates were incubated for 5 to 7 days in a BOD incubator at a temperature of 25±2 ^{0}C. The identification of the fungi was carried out by examining the microscopic morphological characteristics and the macroscopic features of their colonial growth (Ellis, 1971; Barnett and Hunter, 1972 &1995; Adhikari *et al.,* 1996; Watanabe, 2010). The total count of the two stages was determined and reported as colony-forming units per cubic meter of air (CFU m^{-3}). The identification of fungal colonies was carried out in consultation with the Department of life sciences, Manipur University.

Results and Discussion

Contamination of airborne fungi in the schools

The results of the investigation showed the occurrence of seven commonly isolated fungal genera, namely, *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium*, and *Rhizopus* in the schools. The identified genera, along with the unidentified fungi, were further investigated to quantify their concentrations in the air. The occurrence of fungi in the air showed both spatial and temporal variation (Table 1).

Out of the five schools, the lowest airborne fungal concentration was observed in the school (S-3). The school (S-3: Ananda Singh Higher Secondary Academy) is situated near the residential area. The fungal concentration obtained in the indoor and outdoor sampling in the S-3 was 465.96 CFU m^{-3} and 543.62 CFU m^{-3}, respectively. The highest fungal concentration in the air was isolated in the school (S-1: Johnstone Higher Secondary School), located in the city center. The fungal concentration in the indoor and outdoor air in the S-1 was 587.75 CFU m^{-3} and 645.99 CFU m^{-3}, respectively. The compara-

tively higher fungal contamination in the S-1 might have been attributed partly to the prevailing microenvironment within the school campus and partly influenced by the environmental setup beyond the campus. The woody vegetation stand and the shady microenvironment in and around the campus might have supported congenial spots for the growth of fungi. Moreover, the presence of wetland (Kangla *Paat*) and rich greenery in the recreational park and the historical sites near the school campus might have also contributed to the fungal load. Importantly, all the studied schools, including the S-1, are naturally ventilated, and the bioparticles from outdoor sources could have easily seeped into the classrooms.

Monthly variations of fungal concentration

The monthly variations in the airborne fungal concentrations in the five schools are presented in Figure 1. In general, it was observed that the fungal concentrations were lower during the two peak winter months (December and January) compared to the months of November and February. The overall average airborne fungal concentrations in the classroom in the four months was 597.28 CFU m^{-3} (November), 482.90 CFU m^{-3} (December) 447.60 CFU m^{-3} (January), and 632.58 CFU m^{-3} (February). Corresponding average airborne fungal concentration in the outdoor air was 670.70 CFU m^{-3} (November), 495.61 CFU m^{-3} (December) 522.44 CFU m^{-3} (January), and 714.47 CFU m^{-3} (February). The variation in the fungal concentrations in the indoor air goes almost in tandem with the outdoor concentrations except that the lowest outdoor concentration felled during December and for indoor during January. There was a significantly positive correlation between the indoor and outdoor airborne fungal concentrations ($r = 0.99$ at $p < 0.001$) in the schools. As per the Commission of the European Communities (CEC), an airborne fungal level < 50 CFU m^{-3} is considered very low; < 200 CFU m^{-3} is considered low; < 1000 CFU m^{-3} is considered intermediate; < 10,000 CFU m^{-3} is considered high in houses (Kuen, 2020). With reference to the above CEC standards for the non-industrial indoor environments, the fungal load observed during the current study may be considered as intermediate in concentration.

Diversity of the Fungal Species

Comparative analysis of the airborne fungal species in the classrooms revealed that the dominant species were that of *Cladosporium* (38.24%), followed subsequently by *Aspergillus* (15.29 %), *Curvularia* (11.76 %), *Alternaria* (10.20%), *Penicillium* (9.08 %), *Fusarium*

Table 1. Indoor and outdoor fungal concentrations in the five schools

Genera	S–1	S–2	S–3	S–4	S–5	All schools
			Indoor (in CFU m^{-3})			
Alternaria	58.25 (09.91)	58.25 (10.39)	51.19 (10.98)	67.07 (11.80)	40.60 (07.81)	275.34 (10.20)
Aspergillus	88.25 (15.01)	81.19 (14.49)	68.84 (14.77)	91.78 (16.15)	82.96 (15.96)	413.01 (15.29)
Cladosporium	227.69 (38.74)	225.92 (40.31)	160.62 (34.47)	210.04 (36.96)	208.27 (40.06)	1032.53 (38.24)
Curvularia	67.07 (11.41)	61.78 (11.02)	56.48 (12.12)	68.84 (12.11)	63.54 (12.22)	317.70 (11.76)
Fusarium	45.89 (07.81)	45.89 (08.19)	42.36 (09.09)	47.66 (08.39)	35.30 (06.79)	217.10 (08.04)
Penicillium	52.95 (09.01)	49.42 (08.82)	47.66 (10.23)	49.42 (08.70)	45.89 (08.83)	245.34 (09.08)
Rhizopus	19.42 (03.30)	15.89 (02.83)	08.83 (01.89)	12.36 (02.17)	17.65 (03.39)	74.13 (02.75)
Unidentified	28.24 (04.80)	21.18 (03.78)	30.01 (06.44)	21.18 (03.73)	24.71 (04.75)	125.32 (04.64)
Total	587.75 (100)	559.51 (100)	465.96 (100)	568.33 (100)	518.91 (100)	2700.45 (100)
			Outdoor (in CFU m^{-3})			
Alternaria	67.07 (10.38)	79.43 (13.20)	68.84 (12.66)	70.60 (11.43)	51.19 (08.61)	337.12 (11.22)
Aspergillus	95.31 (14.75)	93.55 (15.54)	75.90 (13.96)	100.61 (16.92)	100.61 (16.91)	465.96 (15.51)
Cladosporium	231.22 (35.79)	188.86 (31.38)	178.27 (32.79)	213.57 (34.57)	217.10 (36.50)	1029.00 (34.25)
Curvularia	79.43 (12.30)	74.13 (12.32)	67.07 (12.34)	81.19 (13.14)	70.60 (11.87)	372.42 (12.40)
Fusarium	51.19 (07.92)	56.48 (09.38)	49.42 (09.09)	54.72 (08.86)	45.89 (07.72)	257.69 (08.58)
Penicillium	65.31 (10.11)	61.78 (10.26)	52.95 (09.74)	47.66 (07.71)	60.01 (10.09)	287.70 (09.58)
Rhizopus	24.71 (03.83)	19.42 (03.23)	14.12 (02.60)	19.42 (03.14)	19.42 (03.26)	97.08 (03.23)
Unidentified	31.77 (04.92)	28.24 (04.69)	37.07 (06.82)	30.01 (04.86)	30.01 (05.04)	157.09 (05.23)
Total	645.99 (100)	601.87 (100)	543.62 (100)	617.75 (100)	594.81 (100)	3004.03 (100)

**Figure in the parenthesis gives the percentage contribution of the respective genera*

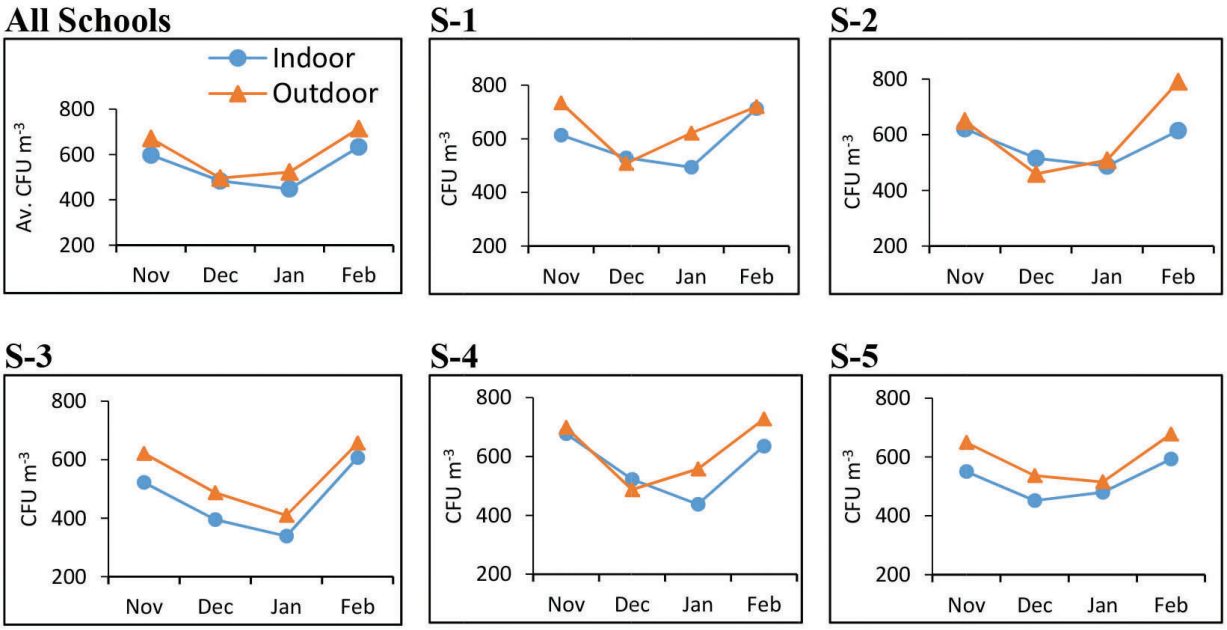

Fig. 1. Monthly variation of fungal concentrations (November 2018 to February 2019)

(8.04 %) and *Rhizopus* (2.75 %). The corresponding order of species dominance in the outdoor sampling was *Cladosporium* (34.25%), *Aspergillus* (15.51%), *Penicillium* (12.40 %), *Alternaria* (11.22%), *Fusarium* (9.58%), *Curvularia* (8.58%), and *Rhizopus* (3.23%). Some of the dominant species recorded in the study are *Aspergillus niger, Aspergillus flavus, Cladosporium cladosporioides, Penicillium citrinum, Alternaria alternata,* and *Curvularia lunata*. Besides these species, several sterile mycelium colonies were also isolated during the study. Previous studies have reported changes of dominant fungal species with the

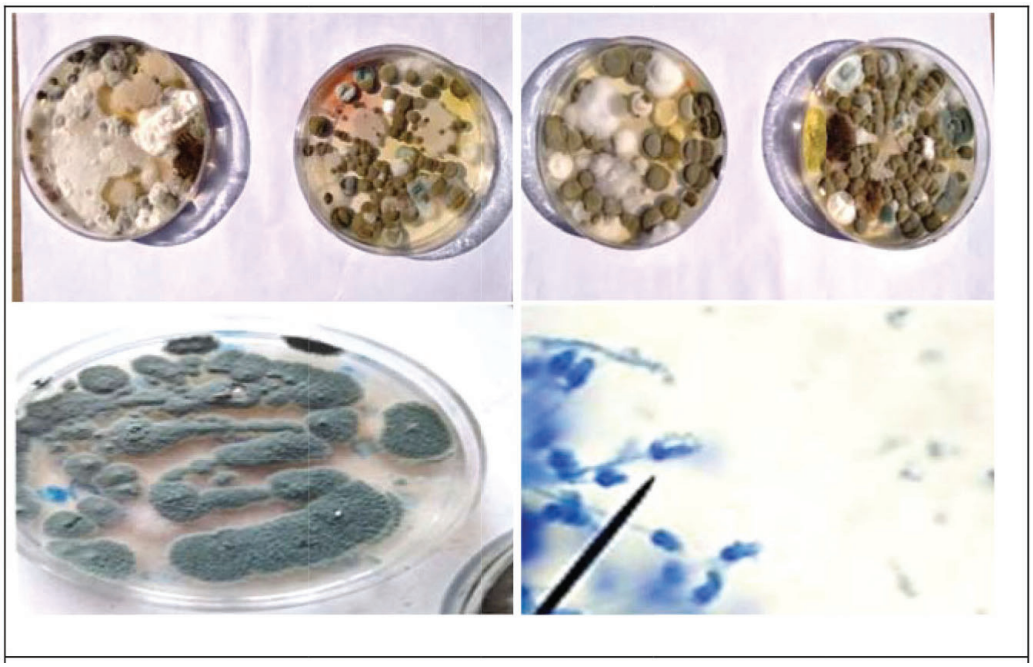

Fig. 2. Fungal colonies, isolated from: indoor air (top-left) and outdoor air (top-right). *Penicillium citrinum* in culture medium (bottom-left) and microscopic view of an isolated fungi (bottom-right)

season. For instance, a study by Singh *et al*. (1995) concluded the dominance of *Penicillium* in autumn and *Cladosporium* in winter. In this study, *Cladosporium* was the dominant species isolated from both the indoor and outdoor environments. In an aerobiology study in America, Shelton *et al.* (2002) concluded that *Cladosporium, Penicillium, Aspergillus,* and *Alternaria* were the widespread outdoor and indoor fungi. The dominating airborne fungi in schools and higher institutions mostly belonged to the genera *Cladosporium,* followed by *Penicillium Aspergillus, Alternaria, Fusarium, Rhizopus,* and *Curvularia* (Uzochukwu and Nkpouto, 2013; Reddy and Srinivas, 2017).

Aspergillus and *Penicillium* are well-known opportunistic fungal pathogens and are often associated with allergy, aspergillosis, rhinitis, asthma, and conjunctivitis, presenting potential candidates for sick building syndrome (Schwab and Straus, 2004). Sekulska *et al.* (2007) and Mousavi *et al.* (2016) reported the occurrence of allergenic and toxigenic bio-contaminants in institutions and working environments. Human pathogens belonging to *Cladosporium* and *Alternaria* are well-known to have adverse effects on children's health in classrooms (Bush and Prochnau, 2004; Sekulska *et al.*, 2007). Hence, the presence of the airborne fungal species belonging to *Cladosporium, Alternaria, Aspergillus,* and *Penicillium* in the classrooms in the current study is a cause of concern.

Indoor-to-Outdoor (I/O) Ratio

The I/O ratio was used to evaluate whether there was an indoor fungal source. The calculated values of the I/O ratio for all the schools were less than one. The values of the I/O ratio in the five schools were 0.91 (S-1), 0.93 (S-2), 0.86 (S-3), 0.92 (S-4), and 0.87 (S-5). The obtained I/O ratio points out that the airborne fungal contaminants in the classroom have origin from exogenous sources. Many studies (Tsai *et al.*, 2002; Tsai and Macher, 2005; Yafetto and Adator, 2018; Karmakar *et al.*, 2020) have reported bio-contamination of indoor air by exogenous fungal sources.

Conclusion

This investigation showed that the outdoor concentrations of fungal aerosols were greater than indoor concentrations in all the schools, indicating an external source of indoor contamination. The dominant airborne fungal species isolated in the study belong to *Cladosporium, Aspergillus,* and *Penicillium*. Among these fungal genera, *Cladosporium* contributed the maximum airborne fungal concentration. Some of the isolated fungi in the current study, *viz.*, the *Aspergillus, Penicillium*, *Alternaria*, and *Fusarium*, are toxic to humans and, thus, may pose serious health threats to the students and teachers in the schools. However, due to time limitations, the current study fells short of depicting a holistic picture of the fun-

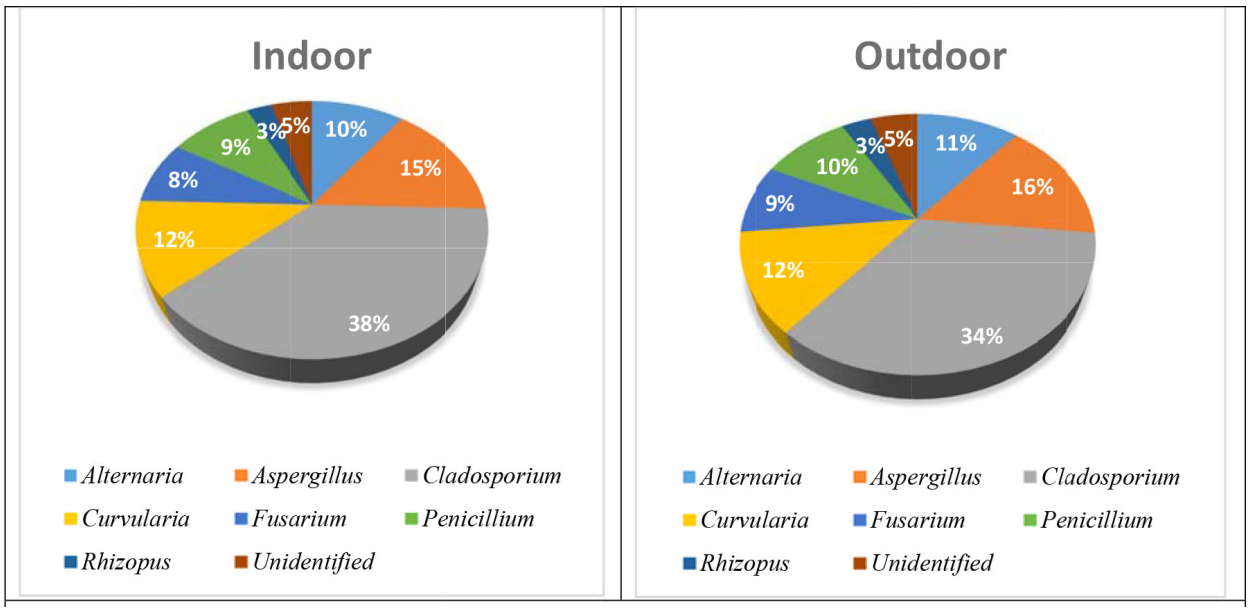

Fig. 3. Genera-wise fungal distribution in the indoor and outdoor air

gal contamination in the schools. Nevertheless, the finding of the study is significant in revealing the state of IAQ in the urban schools in Imphal with respect to one of the prevalent biological contaminants.

Acknowledgments

The authors are grateful to the Directors of Education (S) Imphal, Manipur, for permitting the study. We also thank the technical staff working on aerobiology in the Department of Life sciences, Manipur University, for the technical guidance during the study.

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