ELUCIDATION OF INDOOR AIR MICROBIAL AIR QUALITY AND OCCURRENCE OF MYCOTOXIN IN OLD BUILDING

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by

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LIST OF SYMBOLS

%	Percent
g	Gram
m	Metre
m^3	Cubic metre
ng	Nano-gram
mL	Milli-litre
ppm	Parts per million
sec	Second
min	Minute
°C	Degree Celcius
%	Percent

LIST OF ABBREVIATIONS

ACMV	Air-Conditioning and Mechanical Ventilation Systems
AFB1	Aflatoxin B1
AFLs	Aflatoxins
ASHRAE	American Society of Heating, Air Conditioning & Engineers
BLAST	Basic Local Alignment Search Tool
BRI	Building-related illness
CAST	Council For Agricultural Science and Technology
CCCs	Childcare Centres
CO	Carbon Monoxide
CONTC	Concentration of No Toxicology Concern
CO2	Carbon Dioxide
Di	Inhaled daily dose
DOSH	Department of Safety and Health
EHRC	Environmental Health Research Centre
EPA	Environmental Protection Agency
GBI	Green Building Index
HVAC	Heating, Ventilating, and Air-Conditioning
IARC	International Agency for Research on Cancer
IAQ	Indoor Air Quality
IEQ	Indoor Environment Quality
ICOP	Industry Code of Practices
IMR	Institute for Medical Research
ISHAM	International Society of Human and Animal Mycology
MEA	Malt extract agar
MICOP	Malaysia Industry Code of Practices
MOH	Ministry of Health
NCBI	National Centre for Biotechnology Information
NIOSH	National Institute for Occupational Safety and Health
NV	Natural Ventilation
PDA	Potato Dextrose Agar
RH	Relative Humidity

SBS	Sick Building Syndrome
SDA	Saboraud Dextrose Agar
SPSS	Statistical Package for Social Science
STE	Sterigmatocystin
TVOC	Total Volatile Organic Compounds
US EPA	United States Environmental Protection Agency
USM	Universiti Sains Malaysia
VOC	Volatile Organic Compounds
WHO	World Health Organization

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PENJELASAN KUALITI UDARA DALAMAN MIKROB DI DALAM UDARA DAN KEJADIAN MIKOTOKSIN DI BANGUNAN LAMA

ABSTRAK

Mikotoksin ialah metabolit sekunder yang dikeluarkan oleh kulat berfilamen dan kulapuk yang boleh memberi kesan negatif kepada kesihatan penghuni bangunan. Kebanyakan penghuni bangunan cenderung menghabiskan masa mereka di dalam persekitaran dalaman, yang mungkin berisiko kesihatan akibat kehadiran mikotoksin, sebagai produk sampingan kulat. Di samping itu, bangunan lama sering mengalami isu penting akibat kemerosotan bahan dan fizikal, yang boleh membawa kepada kehadiran bahan cemar biologi berbahaya dalam persekitaran penghuni. Di Malaysia, pada ketika ini, terdapat kajian yang terhad mengenai kejadian mikotoksin dalam udara dalaman, terutamanya di bangunan lama. Kajian ini menyiasat dan mengenal pasti kulat dan mikotoksin yang berlaku di bangunan lama terpilih, bersama-sama dengan potensi risiko kesihatan di kalangan penghuni bangunan. Dua bangunan lama iaitu Institut Penyelidikan Perubatan (IMR) dan Universiti Sains Malaysia (USM) telah dipilih dalam kajian ini. Terdapat 18 lokasi persampelan, dan setiap bangunan mempunyai sembilan lokasi yang dikenal pasti dengan masalah kulat dan kerosakan lembapan. Jumlah penghuni bangunan yang menyertai tinjauan soal selidik adalah 49 orang, iaitu 25 adalah penghuni bangunan dari IMR, dan 24 adalah penghuni bangunan dari bangunan USM. Keputusan menunjukkan bahawa 17 daripada lokasi persampelan di bangunan lama tidak mematuhi had yang disyorkan yang dinyatakan oleh garis panduan Industry Code of Practice, Indoor Air Quality (ICOP, IAQ) Malaysia Department of Safety and Health DOSH (2010). Secara keseluruhan, lokasi BS3 sahaja yang mematuhi had kepekatan CO_2, peratusan kelembapan dan bacaan suhu yang disyorkan. Sebanyak 333 spesies telah diasingkan daripada tiga jenis sampel

(udara dalaman, sapuan swab dan kikisan) dan berjaya dikenal pasti menggunakan pengenalpastian molekul. Unit pembentuk koloni dari 18 lokasi persampelan berada dalam julat 215.00 – 5235.71 CFU/m^3. Empat lokasi persampelan dilaporkan tidak mematuhi garis panduan yang ditetapkan oleh ICOP IAQ DOSH 2010, dengan nilai yang lebih tinggi daripada 1000 CFU/m3. Kebanyakan lokasi persampelan menunjukkan kehadiran mikotoksin, kecuali di AS5 di bangunan IMR. Sampel bahan binaan mempunyai tahap mikotoksin yang lebih tinggi daripada yang berpunca dari udara dalaman, dan tahap tertinggi sterigmatocystin 29.6130 ng/g dan aflatoksin 37.7358 ng/g dilaporkan di USM. Dos pernafasan harian (Di) AFB_1 dan sterigmatocystin dianggarkan pada kepekatan tertinggi kedua-dua mikotoksin ialah 0.0670 ng/kg bw/day dan 0.0142 ng/kg bw/day, masing-masing. Penemuan ini jauh lebih rendah daripada nilai ambang ConNTC 30 ng/m^3. Mengikut kaedah penilaian risiko, tiada ancaman ketara terhadap kesihatan penghuni bangunan.

ELUCIDATION OF INDOOR AIR MICROBIAL AIR QUALITY AND OCCURRENCE OF MYCOTOXIN IN OLD BUILDING

ABSTRACT

Mycotoxins are secondary metabolites released by filamentous fungi and moulds that may negatively impact the building occupant's health. Most of the building occupants tend to spend their time in an indoor environment, which might be a health risk due to the presence of mycotoxins, by-products of fungi. In addition, older buildings often experience significant issues due to material and physical deterioration, which can lead to the presence of harmful biological contaminants in the occupants' environment. In Malaysia, at this point, there has been limited study on the occurrence of mycotoxins in indoor air, especially in old buildings. The present study investigated and identified the fungi and mycotoxins that occurred in selected old buildings, together with the potential health risks among the building occupants. Two old buildings, the Institute for Medical Research (IMR) and Universiti Sains Malaysia (USM), were selected for this study. There were 18 sampling locations, and each building had nine locations that were identified with the fungus problem and moisture damage. The total number of building occupants who joined the survey using a validated self-administered questionnaire was 49, of which 25 were building occupants from IMR, and 24 were building occupants from the USM building. Results indicated that 17 of the sampling locations in old buildings were observed not to comply with the recommended limit stated by the ICOP IAQ Malaysia DOSH (2010) guideline. Overall, only one location, BS3, complied with the recommended limit of CO_2 concentration, humidity percentage, and temperature reading. A total of 333 species were isolated from three types of samples (indoor air, swab, and scrap) and successfully identified using molecular identification. The colony-forming units from

18 sampling locations were in the range of 215.00 – 5235.71 CFU/m^3. Four sampling locations were reported as not complying with the guideline set by ICOP IAQ DOSH 2010, with a higher value than 1000 CFU/m^3. Most of the sampling locations showed the presence of mycotoxin, except at AS5 in the IMR building. Building material samples had higher mycotoxin levels than those from indoor air, and the highest levels of sterigmatocystin 29.6130 ng/g and aflatoxin 37.7358 ng/g were reported in USM. Daily inhaled doses (Di) of AFB_1 and sterigmatocystin estimated on the highest concentration of both mycotoxins were 0.0670 ng/kg bw/day and 0.0142 ng/kg bw/day, respectively. These findings were much lower than the CoNTC threshold value of 30 ng/m^3. According to the risk assessment method, there was no significant threat to the health of the building occupants.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The airborne environment significantly impacts human well-being, positive or negative, as most people spend more time indoors, particularly in buildings. Most of the research on indoor environments has noted that indoor air pollutants can be two to five times higher than outdoor pollutants. They highlighted that 90% of people spend their time in an indoor environment (ASHRAE-55, 2017; Bloom, 2008; Frontczak et al., 2012; Mousavi et al., 2016; Nguyen Thi et al., 2005). The poor indoor air quality can be seen in the health issues among building occupants (Polizzi et al., 2009). The presence of microbiological contaminants, especially fungi, negatively affected the health of people living in indoor buildings (Hardin et al., 2009; Sham et al., 2021; Brown et al., 2012). Building occupants will face severe invasive infections caused by these microorganisms (Brown et al., 2012). These, in turn, weaken the immune system and possess a range of toxic effects, including carcinogenic, mutagenic, embryotoxic, teratogenic, hemorrhagic, dermatotoxic, cytotoxic, neurotoxic activities, and impair fertility (Boonen et al., 2012; Bünger et al., 2004; Curtis et al., 2004; Jarvis & Miller, 2005; Jezak et al., 2016).

Notably, old buildings frequently face challenges with preservation and maintenance issues, decaying infrastructure, inadequate ventilation systems, and moisture issues, which encourage fungi proliferation (Azizi et al., 2016). According to Oliveira et al. (2020), most old buildings were at an advanced stage of degradation and require specialized knowledge to sustain the old building features. Fadilah & Juliana (2012) also highlighted that old buildings tend to have more occupants' complaints due to poor indoor air quality, inadequate Heating, Ventilation, and Air Conditioning

(HVAC), and uneven air distribution. Thus, under these conditions, indoor fungi or bioaerosols will settle on various surfaces and rapidly proliferate, especially in areas with poor ventilation and damp building environments (Dyląg, 2017; Jarvis & Miller, 2005). This represents a major concern due to the production of secondary metabolites or mycotoxins, which can expose building occupants to health risks, particularly through inhalation and dermal contact (Peraica et al., 1999). Generally, the most abundant genera identified in indoor buildings and associated with health issues were *Alternaria, Aspergillus, Cladosporium*, and *Penicillium* (Hung et al., 2011; Su et al., 2001; Uzochukwu & Nkpouto, 2013). Among these, *Aspergillus versicolor*, which was detected in building material, was known to produce sterigmatocystin (STE), a Group 2B carcinogen classified by the International Agency for Research on Cancer (IARC) (Jarvis & Miller, 2005). For instance, STE was recognized biogenic precursor of aflatoxin B_1 (AFB_1), sharing notable structural and biological similarities (Zingales et al., 2020). Recently, Rahman et al. (2024) identified STE in buildings, which was found to be produced by fungi of the *Aspergillus* genus. According to Singh (2000), older buildings are more prone to fungal proliferation due to decayed building structures. Rahman et al. (2012) reported that fungal proliferation in the building served as an indicator of poor indoor air quality (IAQ), and the building was subsequently classified as a “sick building”. Therefore, studies of older buildings were significant, as their structural conditions may contribute to poor IAQ and pose a potential health risk to building occupants.

Two mycotoxins, named AFB_1 and STE, were the most secondary metabolite toxins found, which are related to the building environment (Bloom, 2008; Cai et al., 2011; Navale et al., 2021; Nguyen Thi et al., 2005). However, most studies of mycotoxin exposure were commonly associated with ingestion rather than inhalation, and to date, there are no regulatory limits established for airborne mycotoxins (Bryden, 2007; Gbashi et al., 2020; Joshi et al., 2022; Kabak, 2016; Leong et al., 2010, 2012; Magan & Olsen, 2004; Omotayo et al., 2019; Tahoun et al., 2021). In a study by Schlosser et al. (2020), they used an indirect risk assessment method as benchmark values for mycotoxin exposure levels, the concentration of no toxicological concern (CoNTC), which was derived by Drew & Frangos (2007). According to Hallak et al. (2023), occupants of buildings, particularly those working in indoor environments, are at high risk due to the inhalation of hazardous mycotoxins, bioaerosol contaminants, and various microbiological species commonly found in indoor air and on surfaces.

There has been some evaluation of indoor microbiological quality in Malaysia, but research still needs to be done on mycotoxin occurrence, particularly in older buildings. The elevation of health problems related to sick buildings was expected due to our warm and humid weather. To address this issue, this study revealed the prevalence of fungi in indoor air and the fungal contamination on surfaces in two old buildings: the Institute of Medical Research (IMR) and Universiti Sains Malaysia (USM). These locations have a history of reported indoor air quality problems. Prolonged exposure to fungal contamination in indoor environments can lead to “sick building syndrome” (SBS), which includes symptoms such as ocular, nasal, throat, and skin irritation, along with headaches and fatigue (WHO, 1983; Norbäck et al., 2016). For this reason, sociodemographic characteristics of the environment, and IAQ parameters were collected to collect data about background information, workplace details, and health status. These findings were aligned with the results of mycotoxin identification obtained through Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS).

1.1.1 Problem statement and rationale of the study

Fungal contamination in indoor environments has gained interest due to its potential impact on human health, especially in buildings with poor maintenance and inadequate ventilation. Among these, old buildings represent a significant area of concern, as they are often prone to structural deterioration, water intrusion, and high relative humidity, which creates an ideal environment for fungal proliferation (Singh, 2000). Moreover, fungi produce secondary metabolites, particularly mycotoxins, which are a significant concern due to their potential health risks to building occupants. According to Jezak et al. (2016), *Aspergillus* and *Penicillium* are the primary fungal genera found in the indoor environment that produce mycotoxins harmful to human health. A review of the fungi diversity and their effect on the building material and occupants has been carried out by Kumar & Verma (2010). They found that fungal infections that pose no threat to healthy individuals can be fatal to those suffering from immunodeficiency with a compromised immune system. These facts were also supported by Azlan et al. (2022), where the presence of fungal contamination poses a potential health risk to building occupants, which may consequently reduce their work performance and productivity.

The problem of fungal pollution was familiar in Malaysia, a tropical country with warm and humid weather favouring fungi growth (Cai et al., 2011; Fadilah & Juliana, 2012; I. et al., 2009; Onwusereaka et al., 2022; Rahman et al., 2012; Xin et al., 2021; Yau et al., 2012; Yogeswaran et al., 2023; Zainal et al., 2019). According to Ahmad et al. (2025), buildings with irregular maintenance and outdated heating, ventilation, and air conditioning (HVAC) systems contributed to the spread of fungal spores and inadequate air ventilation in indoor environments. To our knowledge, there was no detailed identification of mycotoxins or fungi in the indoor air of older building environments currently available. By evaluating information collected from building occupants with analytical findings obtained through LC-MS/MS, this study contributes to a clearer understanding of the potential health risk of indoor fungal exposure. In addition, the highlighted strength of this study was the old building, which is frequently overlooked in indoor air quality assessments despite being highly susceptible to microbial contamination due to its age, decay structures, and persistent dampness. This study will provide valuable information and support evidence for the development of policies, workplace safety standards, and preventive actions in building maintenance and environmental health.

The occupants of the building deserve a healthy indoor air quality (IAQ) environment conducive to work activities. Research conducted by Rahman et al. (2012) reported on excessive fungal growth in the USM building (the University's clinic), Pulau Pinang, which was established in 1969, making it over 50 years old. As a result, the building was closed and declared unfit for occupancy. As the remediation progressed, the building was successfully restored and no longer exhibited characteristics related to a sick building. Therefore, addressing fungal contamination is significant to ensure a safe indoor environment. A study by Xin et al. (2021) found that the building occupants in USM Pulau Pinang were unaware of fungal contamination and IAQ. They also found, some sampling sites in the USM building had a higher fungal load that exceeded the recommended levels set by the Malaysian Industrial Code of Practice (MICOP). Additionally, 11 fungal genera were identified, including *Aspergillus, Penicillium,* and *Cladosporium*. Despite the presence of fungal contamination, the mycotoxins associated with the fungi were not reported. Inspired by the study, the present study was planned and carried out to investigate buildings in USM that might have repeated issues of mould growth and dampness. In addition, this study aimed to identify the presence of mycotoxins of AFB_1 and STE, potentially produced by the airborne fungus in the indoor environment. Data collected from this study will enhance the awareness among building occupants in the USM building regarding the visibility of fungus. A study by Ahmed et al. (2018) had suggested a series of practical that can be implemented to improve IAQ. One of the recommendations was a regular inspection and maintenance regime for the building.

The Institute of Medical Research (IMR), in the Federal Territory of Kuala Lumpur, on the west coast of Peninsular Malaysia, was selected as the second oldest building due to informal reports and anecdotal feedback from building occupants indicating concern about IAQ issues. Furthermore, the IMR building was established in 1901, which is approximately 124 years old. Thus, the duration of long-standing, established IMR buildings provides a valuable opportunity to study the impact of building age on indoor microbial levels and the potential health risks for occupants.

1.1.2 Scope of study

This study's scope covers identifying fungus species and mycotoxins that occurred in 18 sampling locations from both old buildings: the USM, Pulau Pinang, and the IMR, Kuala Lumpur. In addition, a survey on building occupants using modified questionnaires from the Industry Code of Practice Indoor Air Quality Malaysia (ICOP IAQ., 2010) was conducted to evaluate the perception of indoor air quality and experiences of sick building syndrome (SBS). This study also covers the measurement of physical parameters and indoor contaminants such as the temperature, humidity, and concentrations of carbon dioxide (CO_2). Collected data is then compared to the IAQ guidelines by ICOP (2010).

1.1.3 Significance of the study

The longer a building is constructed, the more damage will eventually show up because of deterioration over time (Safe Work Manitoba, 2015). Older buildings were noted to have worse conditions. At worst, biodeterioration issues from poor indoor quality or indoor microbial contaminants lead to more harm than the deterioration of buildings (Ahmed et al., 2018). Realizing that many areas within the USM and IMR building are actively utilized by occupants for various purposes, particularly in research and laboratory work. It was necessary to protect the environment from fungal contamination to ensure the health of occupants and the integrity of ongoing scientific work. The output of this research will provide valuable information to help improve indoor air quality and create more comfortable workspaces for building occupants. Therefore, this finding will encourage the management of USM and IMR to enhance the ventilation system, control moisture, conduct regular cleaning, and utilize antimicrobial materials, which can prevent microbial development in the building environment. The identification of fungal contamination data will raise awareness among building occupants, particularly those who may have previously disregarded its presence. Hence, building occupants should be aware to promptly report to the building management team any visible signs of mould, address factors influencing fungal growth, maintain cleanliness in their personal work areas, prevent moisture accumulation, and support preventive measures.

1.1.4 Research question

Research questions in this study were:

i. What are the fungus genera/species to be detected in the indoor and building materials of these old buildings?

ii. What are the levels of the indoor air parameters (the humidity, temperature, and concentration of carbon dioxide) in the old buildings that could affect the growth of fungus?

iii. How could various fungus genera/species impact the occurrence of mycotoxins in old buildings?

iv. What are the potential health effects among occupants of these old buildings?

1.1.5 Limitations of the study

This study had several limitations. Some data were missing due to the self-administered survey method. In certain questions, such as background information, respondents opted not to answer because of privacy concerns. As a result, the survey was anonymous and could not follow up with the respondents to complete the missing data. In addition, some sampling locations have limited building occupants because not all the staff were allowed to be present due to the COVID-19 pandemic. Limited indoor air and building materials were collected due to insufficient facilities and funding.

1.2 Research objective

This study aims to identify and determine mould and mycotoxin contamination in the indoor air of two selected old buildings (USM and IMR). The specific objectives are to identify the fungi species that dominate the old buildings. During the inspection, this study examined intrinsic factors related to the building, including design details, how people are exposed to various conditions, their comfort levels, possible sources of contamination, and the state of ventilation and air conditioning. This study also measured extrinsic factors like temperature, humidity, and carbon dioxide levels. Furthermore, the association of these indoor parameters with the demographic and health effects among building occupants is determined.

CHAPTER 2

LITERATURE REVIEW

2.1 Indoor air quality

2.1.1 Indoor air pollutant

Most people prefer an indoor environment to an outdoor one. A study conducted by Zainuddin (2022) revealed that 58% of adults, 90% of children, and 100% of babies spend their time indoors. Aside from residential homes, there were other indoor spaces such as offices, commercial and industrial structures, educational facilities (schools and universities), and others (Mannan & Al-Ghamdi, 2021). IAQ was described as the air quality inside buildings and structures. Hence, the reflection of the good or poor IAQ will affect the physical and psychological well-being of building occupants (DOSH, 2010). According to Azlan et al. (2022), inadequate IAQ triggers SBS, which significantly influences health symptoms like headaches, fatigue, strained eyes, wheezing, dry throat, coughing, stress, decreased concentration, itchy skin, nausea, and stomach pain. Therefore, it is significant to actively manage indoor air quality by monitoring and controlling the parameters such as humidity, temperature, ventilation, and pollution levels (U.S. EPA, 1997).

Indoor air pollutants were one of the impacts of neglecting the importance of controlling indoor air quality. As it poses severe dangers to human health and well-being, understanding the causes, characteristics, and effects of this problem is crucial. The potential sources of indoor air pollution include building design and sealing (such as airtight construction that leads to the build-up of pollutants indoors), furnishing, cleaning and hygiene products, air fresheners, electronic equipment, and activities of building occupants (such as cooking, smoking, and the use of appliances or equipment) (Guideline stated in U.S. EPA 2008). Inadequate ventilation in indoor environments impedes air exchange between indoor and outdoor environments. Furthermore, pollutants from vehicle emissions, industrial emissions and outdoor allergens were also sources of pollution that could be considered infiltrated in indoor areas. Health issues from indoor air pollution can be seen or diagnosed shortly after multiple exposures. Examples of common and treatable issues in the short term are eye, nose and throat irritation, headaches, dizziness, and fatigue.

Buildings are frequently equipped with air conditioning and mechanical ventilation (ACMV) systems to create comfortable working environments and cosy residences. However, the consumption of air conditioning can enhance global warming and limit the fresh air entering the building (Daghigh, 2015; Sharma et al., 2022). Limiting fresh air indoors would negatively impact human health due to poor indoor air quality. Thus, the reflection of poor indoor air quality on building occupants' health can be observed. In addition, nosocomial fungal infection is one of the impacts of poor indoor air quality, specifically in buildings with mould contamination (Raoofi et al., 2023).

Previous research confirmed that biological agents such as *Aspergillus sp.* can be inhaled and may cause various diseases such as invasive pulmonary aspergillosis, aspergilloma, and different forms of hypersensitivity diseases (Greub & Bille, 1998; Latgé, 1999; Mousavi et al., 2016). A study by Schlosser et al. (2020) highlighted that to evaluate health risk, the daily inhalation dose of building occupants must undergo a health risk assessment, which compares the level of exposure to airborne mycotoxins with the benchmark value of CoNTC. A study by Singh (2000) stated that fungal contamination can occur in both modern and historic buildings as a result of poor indoor air quality (IAQ). However, the study also noted that the problem tends to be more severe in older buildings, especially those with aged infrastructure, water damage, and irregular maintenance. According to Hasan et al. (2023), buildings that are 100 years old or more are declared as historic buildings, while buildings that are less than 100 years old are declared as heritage buildings. The heritage organization stated that buildings aged 50 years and above in Malaysia were classified as old buildings (National Heritage Act, 2006). As a result, the old building with the poor IAQ was expected to experience a proliferation of fungi with the presence of carcinogenic mycotoxins. The study published by the World Health Organization (WHO) included a list of the 19 fungi that are the biggest threat to public health, making it the first-ever list of fungal "priority pathogens" (WHO, 2022). The fungus was segregated into three groups: the critical priority group, high priority group and medium priority group. *Cryptococcus neoformans*, *Aspergillus fumigatus, Candida auris,* and *Candida albicans* are categorised as the critical priority group. While *Nakaseomyces glabrata* (*Candida glabrata*), *Eumycetoma causative agents*, *Fusarium sp., Candida parapsilosis, Histoplasma sp*., *Mucorales*, and *Candida tropicalis* are categorised as high-priority group. Other fungi, namely *Scedosporium sp., Lomentospora prolificans, Coccidioides sp., Pichia kudriavzeveii (Candida krusei), Cryptococcus gattii, Talaromyces marneffei, Pneumocystis jirovecii,* and *Paracoccidioides spp.* are categorised as medium priority group.

2.1.2 Factor influencing indoor air quality

IAQ was influenced by several factors, including building design. The technical problems related to building maintenance include the building's features, floor, surface materials, ventilation, cleaning process, heating, and cooling systems (DOSH, 2010). The characteristics of the building can be explained in terms of its construction material and architectural design. According to Soltani (2016) sustainable building design impacts occupants and the surrounding environment. Therefore, the design of the building must have significant resistance to moisture problems with the proper maintenance and construction implementation (U.S. EPA, 2013). A review by Hallak et al. (2023) the building materials themselves may contribute to the deterioration of indoor air quality. As time passes, structural flaws will become more noticeable, and they will be most severe during a natural disaster like Hurricane Katrina (Bloom et al., 2009). According to Lax et al. (2019) dampness and mould were the primary issues experienced in most of the buildings in the United States. Similar Malaysian buildings are also constantly exposed to unexpected weather that usually occurs in tropical climate regions (Hassan et al., 2015). The building maintenance commonly deals with issues such as water leaks, microbial growth, and dirt (Wahab et al., 2015). Furthermore, the presence of moisture within building materials or walls can weaken the mechanical strength of the structure and cause damage to any object resting against the wall (Alfano et al., 2023). Dampness can also cause chemical degradation to building materials and produce the emission of volatile organic compounds (VOC), which may become an indication of nasal mucosal swelling and inflammation (Wang et al., 2020). When building materials that are damaged during construction are exposed to excessive moisture, it can further accelerate microbial growth and lead to poor indoor air quality (Kumar & Verma, 2010).

The performance and well-being of building occupants are directly influenced by the ventilation system, which plays a significant role in shaping the indoor air quality within an enclosed space. Without proper ventilation in the enclosed space, the mould will increase and survive, although in conditions of low humidity (Koruba & Piotrowski, 2017). Installation of a ventilation system will help to reduce excessive temperature and enhance the comfort environment within a low temperature (Razak et al., 2020). Inadequate ventilation and accumulation of contaminants on the surfaces of the ventilation, such as plant material dust, pollen, insects, and other pollutants, may also encourage the spread of indoor microbial (Hintika et al., 2009). The outdoor environment has an impact on indoor air quality as well. According to Okten & Asan (2012), microorganisms from the outside can enter an interior environment through a window or a "carrier," such as a building occupant. Therefore, increasing the number of building occupants can increase microbial growth in the building environment. These findings were supported by Beggs (2003), who mentioned that building occupants themselves can be a source of airborne microorganisms, spreading the contaminants through the respiratory droplets, aerosolized microorganisms, and contaminated belongings.

This demonstrated how the presence of fungi in the indoor environment might affect building occupants' health. Building occupants' activities, including pet ownership, plant growth, cooking, insecticide use, incense burning, heating, air conditioning use, and frequent cleaning, were identified as potential sources of pollutants and airborne fungus (Sousa, 2022).

2.1.3 Health effects due to indoor air quality

It is essential to follow the guidelines of DOSH (2010) when maintaining indoor air quality in building environments to ensure compliance with established standards and protect occupant health. The relationship between the building occupant's health status and fungal identification is crucial because the fungi can cause various symptoms and impact the health status (Köhler et al., 2015). Most of the previous studies discussed that children were particularly sensitive to indoor microbes (Jovanovic et al., 2004; Kamaruddin et al., 2015; Hussin, 2011; Norbäck et al., 2016a, 2016b; Onwusereaka et al., 2022; Razak et al., 2020; Sharma et al., 2011). Nevertheless, building occupants were not exempt from the poor indoor environments, which can reduce the physical health and work performance (Braide, W. et al., 2020; Dedesko et al., 2015; Ibrahim et al., 2024; Kumar & Verma, 2010; Laney et al., 2009; Park et al., 2012; Razlin Mansor, 2020; Sousa, 2022). According to Kumar et al. (2007), fungal infections, such as invasive aspergillosis, contribute to life-threatening conditions among immunocompromised individuals due to the difficulty of treatment and high fatality rates. This statement was supported by Bongomin et al. (2017) stated that invasive fungal infection can lead to severe tissue damage and affect most of the human organs among immunocompromised individuals. In 2018, fungal infections were recorded as a silent human killer, with almost 1.5 million cases reported by the Global Fund for Fungal Infections (GAFFI, 2018). Therefore, the condition of building occupants suffering from fungal infections and discomfort due to inadequate maintenance and poor indoor air quality is known as sick building syndrome (SBS) (Daghigh, 2015; Jung et al., 2022; Khan & Karuppayil, 2012; Sham et al., 2021). Building occupants self-diagnosed a variety of health symptoms in a contaminated indoor environment, including sneezing, fatigue, headaches, nausea, disorientation, irritability, and respiratory illnesses, including asthma (Perera et al., 2012). A study conducted in Malaysia found that rhinitis, ocular, throat, cutaneous problems, headaches, and fatigue were common among school children in Johor (Norbäck et al., 2016). In the worst-case scenarios, there was a strong correlation found between the Invasive Aspergillus and the development of the secondary metabolite that causes liver cancer in humans (Claeys et al., 2020; IARC, n.d.; Latgé, 1999; Navale et al., 2021). Other than that, the diseases caused by the fungus included mucous membrane irritation, skin rashes, nausea, immune system suppression, acute or long-term liver damage, acute or long-term central nervous system damage, endocrine impacts, and cancer (Karunasena et al., 2010;

Khan & Karuppayil, 2012; Olsen et al., 1988). Spores, hyphae, and metabolites are categorised as fungal particles, and this relates to fungal infections by Aspergillus, causing allergic lung reactions, severe asthma with fungal sensitization, and allergic fungal rhino sinusitis (Bongomin et al., 2017). This fact reinforced airborne microbes can cause several diseases, varying from mild allergy diseases (more often associated with immunocompetent people) to severe disseminated infections (most often associated with immunocompromised patients (Oliveira et al., 2023). Fungus spreads the disease and may reduce building occupants' productivity. According to Azlan et al. (2022), the performance of building occupants at work is influenced by indoor air quality, and a high-temperature environment can impact cognitive development

2.1.4 Method for assessing indoor air quality

Chemicals frequently contaminated in indoor environments are benzene, carbon monoxide, formaldehyde, naphthalene, nitrogen dioxide, polycyclic aromatic hydrocarbons (especially benzo [α]pyrene), radon, trichloroethylene, and tetrachloroethylene (WHO, 2010). Khan & Karuppayil (2012) provided an explanation of indoor air quality in relation to indoor air microorganisms in their publication on fungal pollution and its control. They stated that the fungal elements of indoor air included volatile fungal metabolites (VFM), (1–3)-β-D glucan, ergosterol, and mycotoxins. Methods for assessing IAQ involved techniques used to measure and analyse pollutants.

Poor handling of airborne pollution and ineffective building maintenance (which included controlling indoor air parameters including temperature, relative humidity, and carbon dioxide) were contributing factors to the facility's inadequate indoor air quality (Norhidayah et al., 2013). Thus, indoor microbiological parameters were measured to determine the environmental factors that contribute to the growth and multiplication of fungus as well as their effects on SBS) According to Zainal et al. (2019), a direct reading device was used to evaluate the real-time measurement of specific indoor air parameters such as carbon dioxide, carbon monoxide, temperature, air movement, relative humidity, formaldehyde, ultra-fine particles, total volatile organic compounds, respirable dust and action level. These indoor air parameters were compared to the guidelines provided by the Department of Health and Safety (DOSH, 2010) in the Malaysian Industry Code of Practice on Indoor Air Quality (MICOP). EVM Environmental monitors (EVM7) were also used to measure indoor air parameters in real time. Azlan et al. (2022) and Azmi et al. (2022) mentioned that their study applied an EVM7-like device to measure indoor air parameters. The EVM7 featured real-time measurement of particulates, selectable sensors for carbon monoxide (CO), hydrogen cyanide (HCN), hydrogen sulphide (H_2S), nitric oxide (NO_2), oxygen (O_2), sulphur dioxide (SO_2), volatile organic compounds (VOC), relative humidity, temperature, and carbon dioxide (CO_2).

On the other hand, indirect measurement was the mathematical method used to identify unknown measurements. Among the instances was the bioindicator, which is characterised as a biological reaction that indicates the existence of contaminants by the manifestation of characteristic symptoms or quantifiable reactions. According to Cabral (2010), fungi can serve as a bioindicator due to the significant influence that SBS has on both the building's structure and its occupants. As a result, fungi can provide valuable insights into the quality of indoor air.

2.1.5 Standard and current guidelines of indoor air quality

The indoor air quality cannot be monitored or maintained without the guidelines; if it is not managed, it will degrade and affect the residents of the building. The Industry Code of Practice on Indoor Air Quality (ICOP IAQ) 2010, issued by the

Department of Occupational Safety and Health (DOSH), was the current guideline for monitoring IAQ assessment and management in workplaces and buildings served by mechanical ventilation and air conditioning (MVAC) systems in Malaysia. Several international organizations have published guidelines on indoor air quality including ASHRAE Standard 62.1, ASHRAE Standard 55, U.S. EPA, and WHO. The American Society of Heating, Refrigerating, and Air Conditioning Engineers published a recommendation titled "Ventilation for Acceptable Indoor Air Quality" in 2019 (ASHRAE 62.1) recommended the ventilation rate and indoor air quality for commercial and institutional buildings, along with guidelines for the ventilation system and equipment. The guideline (ASHRAE-55, 2017), so-called "Thermal Environmental Conditions for Human Occupancy", focused on thermal comfort and guidelines for maintaining appropriate temperature and humidity levels in indoor spaces to ensure occupants' comfort and well-being. The United States Environmental Protection Agency (U.S EPA, 2008) provides guidance on source control, ventilation, and filtration to reduce indoor pollutant exposure and improve IAQ. It also provides specific indoor air pollutants guidelines such as volatile organic compounds (VOCs), Carbon monoxide (CO), Formaldehyde, Particulate Matter (PM2.5), Nitrogen Dioxide (NO_2), and Radon. The Environmental Protection Agency EPA (2021) categorized the effects of pollutants into long-term and immediate impacts. This classification enables building occupants to effectively identify potential sources of pollutants and take appropriate measures to address these issues. Furthermore, the mould prevention and remediation principles are included in the "Mould course". The Environmental Protection Agency (EPA) disclaims that all scenarios and possibly practical procedures or techniques are covered in the course. In 2013, the EPA published "Moisture Control Guidance for Building Design, Construction and Maintenance" and provided helpful guidance on controlling the amount of moisture in architectural structures. The World Health Organization (WHO) was a United Nations organisation that worked to promote health and assist the vulnerable. By complying with the WHO's guidelines, building occupants can thereby preserve their health and achieve the highest level of health attainable. WHO (2009) clarified that the issue of moisture plays a significant role in compromising the quality of indoor air and is considered the driving force behind the proliferation of microbiological pollutants resulting from the presence of moisture.

The "Guidelines for the Registration of Surface Disinfectant" from the National Pharmaceutical Regulatory Agency, which is part of the Ministry of Health (MOH), offers occupants a number of disinfection methods to help lower the likelihood of existing microbes (NPRA, 2025). By following these guidelines, occupants can manage the disinfectant correctly, safely and efficiently. This guideline was recommended for use by healthcare facilities, veterinary areas, food areas, domestic and institutional areas, and industrial areas. This guideline had limitations and exclusion criteria, such as the general cleaning at household, soft/porous surfaces (fabric and leather), products with characteristics rinsed off, products used on medical devices, and sterilizer products (Ultraviolet-C (UVC).

Malaysian Industry Code of Practice on Indoor Air Quality (MICOP), also well known as ICOP IAQ, was relevant to building occupants who were exposed to poor IAQ while working. This standard was used as the current regulation related to IAQ issues (Azlan et al., 2022; Malik et al., 2016; Zaidi et al., 2016; Zainal et al., 2019). ICOP IAQ defined the excellent practices of investigating indoor air quality and listed indoor air contaminants and the maximum limit of exposure. The regulation includes a questionnaire to gather information from building occupants. This information will help determine the connection between the presence of fungus, productivity, and health.

Once the complaint has been submitted, it will be brought to the attention of the building management, and the appropriate individual will conduct a walk-through inspection. Based on the findings of the inspection, the proposed remedy will be implemented, and the issue will be followed up. ICOP IAQ recommends that IAQ parameters should be assessed at regular intervals of eight hours unless otherwise specified. For real-time monitoring, readings were taken every five minutes at each sampling point using a data logging device (EVM-7).

Given the prevailing circumstances of the COVID-19 pandemic, it was imperative to consider the implementation of supplementary guidelines that emphasise the importance of ensuring adequate ventilation in interior spaces to mitigate the potential for viral transmission (WHO, 2020). Consequently, the Ministry of Health (MOH), in collaboration with the Ministry of Human Resources, formed the guidelines focusing on managing indoor air in healthcare facilities due to the COVID-19 pandemic (MOH and MOHR, 2021). Rodrigues & Nosanchuk (2021) stated that Coronavirus (COVID-19) intensified the epidemiology of fungal illnesses, demonstrating that globally transmitted diseases can further complicate an already intricate epidemiological landscape. A summary of the guidelines for indoor air quality is shown in Table 2.1.

Table 2.1 A Summary guideline for IAQ

Parameter	ICOP DOSH (2010)	WHO (2010)	ASHRAE 62.1/55
Ventilation performance indicator			
Carbon dioxide, ppm	C1000 ppm	1000 ppm	1000 ppm
Physical parameter			
Relative humidity, %	40-70%	-	30–60%
Temperature, °C	23-26 °C	-	20–27 °C
Biological contaminant			
Total fungus count, CFU/m^3	1000 CFU/m^3	-	-

Notes:

- C is the ceiling limit that shall not be exceeded at any time. Readings above 1000ppm is indication of inadequate ventilation

2.2 Microbial contamination in buildings

2.2.1 Types of microbial found in an indoor environment

A book entitled "Larone's Medically Important Fungi" provides a comprehensive guide to identifying morphological microorganisms, particularly fungi, that can be observed through direct microscopic examination (Walsh et al., 2018). The book's resources have helped researchers identify and understand indoor contaminants' traits and potential health impacts on humans. Bacteria, viruses, and fungi (including mould) are typical microorganisms in indoor environments, as they can be transmitted by individuals, animals, and the surrounding soil and vegetation (Tran et al., 2020). It was imperative to comprehend the various categories of microorganisms within indoor environments because this knowledge was essential for evaluating the quality of indoor air and the potential health hazards associated with these microorganisms. According to Taubel et al. (2011), bacterial chemicals monactin, nonactin, staurosporin, and valinomycin were found solely in building materials originating from damp structures.

Chloramphenicol, another substance, was more common in household dust and was also responsible for settling airborne particles. The findings also showed the presence of toxic bacteria in indoor samples in addition to mycotoxins. *Streptomyces sp*. produced a bio-active compound and served as a bio-indicator for moisture damage in the area (Huttunen et al., 2004; Hyvärinen et al., 2002). *Streptomyces sp.* have been shown to be Gram-positive, spore-forming, antinobacterial soil organisms that display a wide range of metabolites and valinomycin toxins (Andersson et al., 1996). These findings are supported by the World Health Organization (WHO, 2009). Unicellular prokaryotes were classified by Zabel and Morrell (2020). They represent the primary life forms, characterised by their tiny cell size, typically measuring only a few microns in length. Gram-positive cocci (67.73%), Gram-positive rods (24.26%), and Gram-negative rods (7.10%) were the three types of airborne bacteria that were most frequently observed in buildings and the most frequent genera identified were *Kytococcus* sp., *Micrococcus* sp., *Staphylococcus* sp., *Leifsonia* sp., *Bacillus* sp. and *Corynebacterium* sp. predominated in indoor air.

The bacterial species that exhibited the highest prevalence were *Kytococcus sedentarius, Staphylococcus epidermidis*, and *Micrococcus luteus*. A significant proportion of *Stenotrophomonas maltophilia*, an opportunistic and nosocomial pathogen, was also detected. According to Li et al. (2014), the bacteria found have been increasingly linked to infections in people with weakened immune systems, sometimes resulting in fatal outcomes.

The COVID-19 virus emerged as a prominent subject of interest among researchers. Moreover, in March 2020, the outbreak of COVID-19 was officially declared a pandemic, and it rapidly disseminated as an airborne pathogen (López et al., 2021). The transmission of airborne infectious agents through mucus-to-mucus contact inside indoor environments was a significant pathway (Wei & Li, 2016). According to Ribeiro & Leitão (2017), the respiratory syncytial virus, rhinovirus, metapneumovirus, influenza, parainfluenza virus, human enterovirus, and the development of virus-induced asthma. This association contributed to the onset of respiratory conditions, including pneumonia. Research by Nair et al. (2022) mentioned that the airborne virus could remain infectious in the atmosphere for extended periods. Properly maintaining indoor air quality can minimise the spread of the SARS-CoV-2. Goyal et al. (2011) reported that influenza A, influenza B, and personal identity verification (PIV-1) were notable from the HVAC filters in buildings by Real-time PCR (RT-PCR). The findings of their study suggested that there were notable concentrations of viral aerosols in indoor environments.

Ventilation filter banks can serve as an effective passive sampling device for detecting aerosols. Ventilation filter analysis may be necessary for epidemiology, especially when it comes to the transmission of infectious diseases. Using molecular methods, Myatt et al. (2004) found that airborne picornavirus was present in 32% of the air sampling filters in office buildings. The findings showed a statistically significant positive correlation between the occurrence of virus detection in air filters and the level of building ventilation. This indicated that the average carbon dioxide (CO_2) concentration had surpassed 100 ppm. Other than picornavirus, coronavirus 229E and OC43, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, parainfluenza viruses, influenza, adenoviruses, and respiratory syncytial viruses were also detected in nasal mucus samples.

Mohammad et al. (2021) claimed that 70 genera of fungi were identified in hospital settings in Asia, 45 genera were reported from Europe, 42 genera from Africa, 16 genera from South America, and 12 genera from North America. The findings indicated that fungi posed a significant global concern, especially in the building environment. The primary taxonomy of the groupings was described by Lee et al. (2012). It included the following: Ascomycota, Basidiomycota, Anamorphic or Mitosporic Fungi, Chytridiomycota, Neocallimastigomycota, Mucoromycotina, Kickxellomycotina, Zoopagomycotina, Glomeromycota, as well as Protista and fungal-like organisms. Based on their original published names, the checklist documents 3804 fungi and fungal-like species. *Aspergillus, Fumigatus,* and *Penicillium* were categorised because they were the species that produced the secondary metabolite that was frequently discovered in indoor air (Bünger et al., 2004; CAST, 2003; Sandra et al., 2003; Sorenson, 1999). Other genera like *Alternaria, Rhizopus, Trichoderma,* and *Stachybotrys* also produced mycotoxins (Curtis et al., 2004; Sandra et al., 2003; Sorenson, 1999). A study on water-damaged buildings by Trout et al. (2001) found that *Apergillus, Penicillium*, and *Stachybotrys* were the most prevalent fungi identified in the air sampling. Yogeswaran et al. (2023) reported on findings from laboratory analysis that the most frequent bacteria and fungus identified were *Staphylococcus sp*. (72.2%) and *Cladosporium sp*. (76.7%). Findings from Kwaśna and Kuberka (2020) revealed that the most common genera found in heritage buildings in Poland were *Acremonium, Alternaria, Arthrinium, Aspergillus, Aureobasidium, Botrytis, Chaetomium, Cladosporium, Fusarium, Monodictys, Mucor, Nectria, Oidiodendron, Penicillium, Phoma, Rhizopus, Sarocladium, Simplicillium, Scopulariopsis, Talaromyces, Trichoderma and Tritirachium.* According to Sauliene et al. (2023), in a Lithuanian school building, the most common types of bio-aerosols were found near cabinets and windowsills. Penicillium, Cladosporium, and Acremonium were found to be the most common species in indoor settings. The level of fungi increases along with an increment in the number of students. *Geotrichum, Fusarium, Acremonium, Cladosporium, Leveduras, Penicillium, Alternaria* and *Aspergillus* were the most frequent fungi genera that were determined in the physical characteristics in the room (Sousa, 2022).

In a study by Nielsen (2003), mycological organisms could produce biologically active metabolites. Microorganisms, especially fungi, can generate a substantial quantity of secondary metabolites, which they require for numerous purposes within their native environment. Some fungi that potentially produce secondary metabolites or mycotoxins have been published (Thiboldeaux, 2004). These entities exhibit significant variations in their structural composition and functional characteristics, displaying a wide range of toxicity levels. The fungal genera that were identified to produce mycotoxins were the *Aspergillus, Penicillium, Stachybotrys,* and *Myrothecium*. Bennett and Klich (2003) also reported similar findings, stating that *Fusarium, Penicillium,* and *Aspergillus* were moulds that could also produce mycotoxins. In water-damaged buildings, there were 75 secondary metabolites detected related to the genus, particularly *Penicillium, Aspergillus, Fusarium, Alternaria,* and *Stachybotrys* (Jakšić et al., 2021). Engelhart et al. (2002) mentioned that the fungus of *Aspergillus Versicolor* could produce Sterigmatocystin (STE). These findings were also supported by many other researchers (Bloom et al., 2007; Cigić & Prosen, 2009; Vishwanath et al., 2011). Cigić and Prosen (2009) reported that *Aspergillus* can produce STE and AFs, which explains that STE was a precursor to AFs. Similar to a study by Cressey et al. (2017), STE was described as a precursor of aflatoxin, and its biosynthesis pathway was explained. Arnich et al. (2017) claimed that despite having relatively identical chemical and structural properties, the accumulation of these precursors varies depending on the