Assessment of Indoor Microbiological Air Contamination in Research Facility at University of Malaysia

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Research

Keywords: Indoor air quality, bacteria, fungi, carbon dioxide, temperature and relative humidity

DOI: https://doi.org/10.21203/rs.3.rs-42886/v1

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Abstract

Indoor air quality is a concept that applies to the nature of the environment in and around buildings and facilities in which it contributes to the safety and security of those in the house. The aim of this study was to identify bacterial and fungal species present in the room, determine indoor air quality and investigate consumer views on indoor air quality in the Biology Building, Faculty of Sciences and Technology, National University of Malaysia. This study was conducted at the Biology Building at 8 selected sampling sites. Microbes were sampled using nutrient agar (bacteria) and potato dextrose agar (fungus). All samples of microbes were classified using two approaches; i) morphological examination and ii) biochemical reaction experiments. CO$_2$, temperature and relative humidity are registered using the Direct Sense Monitoring Kit. A survey on customer satisfaction with indoor air quality in the building was carried out and evaluated in order to collect empirical details. This study found that the presence of Bacillus cereus, Bacillus laterosporus, Bacillus sphaericus, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermis, Enterobacter cloacae, Pseudomonas fluarescens, Pseudomonas stuzeri and Aeromonas hydrophila bacterial. The fungi species are Aspergillus niger, Aspergillus nidulans, Penicillium digitatum and Fusarium dimerum. The result also shows that the carbon dioxide, temperature and relative humidity concentrations for most sampling stations comply with the DOSH standards. Moreover, almost all participants reported that their level of health and comfort while in the building is good, while the ventilation system of the building is at a comfortable level. Whereas, the degree of knowledge for most respondents on the value of indoor air quality is high. Two of the recommendations included in this study to enhance indoor air quality are to insure that the air conditioning device is correctly controlled and to raise understanding of the value of indoor air quality among staff and students in the Biology Building.

Introduction

Indoor air pollution, deterioration of indoor air quality by hazardous chemicals and other materials, may be up to 10 times worse than outdoor air pollution. It is because the enclosed areas make it easier for future pathogens to create rather than open spaces (Kong 2015). The enormity of indoor air pollution would be increased in more hazardous buildings such as a chemical plant (Mirmohammadi et al. 2010), a basement car park (Samal et al. 2012), a science lab (Bo et al. 2019) and other industrial buildings (Al-Hemoud et al. 2017). This is attributable to the contaminants generated by these structures, which require different procurement and maintenance, relative to the pollutants released in the natural setting.

More recent focus was given to disruption to museum collections related to indoor pollution contaminants such as formaldehyde, formic acid, acetic acid and chlorinated hydrocarbons (Saraga et al. 2011). Other environmental contaminants (e.g. ammonia, carbon dioxide, arsenic dioxide, nitric acid and peroxyacetyl nitrate) have also been detected in museum settings. Harm done by formaldehyde, nitrogen dioxide, ozone and organic acids has been reported on a broad range of products, including plastics, pulp, textiles and organic colourings. However, there is still a lack of studies on indoor air bacterial and fungal pollution. The production of microorganisms indoor soil is a significant issue with a huge effect on
environmental safety and health. People subjected to large concentrations of microbes in the environment can also acquire allergies. Young adults, particularly graduates, form a significant community of allergy sufferers (Onet et al. 2018; Zhai et al. 2018).

The prevalence of bacteria and fungi in indoor air presents a significant safety and environmental protection issue. In order to define guidelines for indoor air quality protection, it is important to identify the different classes of microorganisms indoors. Indoor air pollution may raise the risk of discomfort, allergic sensitization, acute and chronic respiratory diseases and long-term injury. Exposure to large amounts of bacteria in the soil also contributes to allergies, asthma, hay fever, pneumonia and infections. A significant rise in the amount of allergic responses to fungal spores has been reported in recent years. Young adults, particularly teachers, represent a significant community of allergy sufferers. For this cause, frequent control of indoor air quality in public buildings is entirely justified (Karwoska 2003; Razak & Scribner 2020). Therefore, this study aims to assess the indoor microbiological air contamination in research facility at the university of Malaysia. Evaluation of the indoor microbial contamination was based on the criteria for microbiological cleanliness in interiors submitted by the European Commission in 1993. The environmental factors (carbon dioxide, temperature and relative humidity) of the research facility as well as the degree of satisfaction of the research facility among frequent users have also been observed.

Research Methodology

Sites Description

Research works were carried out in the Biology Building, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), Selangor, Malaysia. This building is located at the main campus of UKM which has been built in 1978 with close and impermeable system. This building has 5 levels in total, with most of it are laboratories for biological researches, lecturer’s office and classrooms. Human activities that occur in this building are involving teaching and learning, research and lab work, etc. It’s been estimated that an average of 502 people has been visited this building in a day in 2018. Those visitors were lecturers, students, researchers, admin workers, and general visitors.

In this study, 8 sampling sites were selected which represent all the functional spaces in the building, such as teaching spaces, research spaces, admin spaces, personal spaces and activity spaces. All of the sampling sites are as follows: i) Biochemistry teaching lab, ii) Molecular Biology Lab, iii) Entomology Lab, iv) Environmental Science Teaching Lab, v) Phycology and Aquatic Biology Lab, vi) Environmental Microbes Lab, vii) Biology Building Main Lobby, and viii) one lecturer office.

Microbes Sampling and Identification

Air sampling for bio-aerosol pollutants such as fungi and bacteria were done by exposing petri dishes close to the source of the ventilation system located at all of the 8 sampling sites. Those petri dishes were exposed for 24 hours. For bacterial sampling, petri dishes with nutrient agar (Oxoid, England)
containing 50 µg/mL of nystatin (anti-fungal) were used, meanwhile for fungal sampling, petri dishes with potatoes dextrose agar (PDA) (Difco, France) containing 5 g/mL of chloramphenicol (anti-bacterial) were used. After 24 hours of air sampling, bacterial samples were sub-cultured into the new nutrient agar and incubated at 37°C for 24 hours. For fungal samples, it also were been sub-cultured into the new PDA and incubated at 30°C for 48 hours.

For bacterial identification, there are two main approaches: a) morphological observation and b) biochemical reaction test. In morphological observation, there were 3 steps have been conducted; i) identification of positive gram Bacillus, ii) identification of positive gram Coccus and iii) identification of negative gram Rod. In the biochemical response test, there were several tests have been conducted such as Catalase test, gram staining, spore staining, starch hydrolysis test, the Voges-Proskauer (VP) test, nitrate reduction test, mannitol fermentation test, glucose fermentation test, motility test, citrate test, coagulase test, novobiocin sensitivity test through the inoculum standardization. For fungal identification, there were two main observation techniques, a) macroscopic and b) microscopic. In macroscopic observation, physical features of fungus colony were observed, meanwhile in microscopic observation, blue methylene was used to color the fungal spore (Bakare et al. 2003), and then the colored fungal spore were observed under the light microscope and recorded. Lastly, all the recorded features of both bacteria and fungus will be analyzed using the Mycology Online of The University of Adelaide (2013).

**Measurement of carbon dioxide, temperature and relative humidity**

The measurement of carbon dioxide (CO₂), temperature and relative humidity were taken at all sampling sites. The tool used to measure these three parameters is Direct Sense monitoring kit, Graywolf (USA). The data was recorded in two sessions, evening and evening. The measurement time taken per session is one hour. Subsequently, the data were analyzed using Wolf Sense PC + ARG and Word Excel software to obtain average readings, standard deviations, minimums, and maximums.

**Survey on User Satisfaction**

In this survey, a total of 32 respondents were selected to answer the questionnaire form to evaluate the user's level of satisfaction towards indoor air quality in Biology Building, Faculty of Science and Technology, UKM. The questionnaire used in this study consisted of four sections, a) Respondents' Background, b) Type of spaces they were exposed, c) Respondents' Perceptions towards Indoor Air Quality in Biology Building and d) Respondents' View towards Indoor Air Quality in Biology Building. Part a) and b) were categorized in nominal value while, part c) and d) were assessed into Likert scale from 1 to 4 (1- strongly disagree, 2-disagree, 3- agree, 4-strongly agree). All data gathered and analyzed descriptively using SPSS software version 23.0.

**Results**
Indoor Bacterial and Fungal Species Isolation

A total of 660 colonies for bacteria and 47 colonies for fungus have been isolated from all 8 sampling sites as shown in the Table 1. There are 9 species of bacteria that have been identified, which are *Bacillus cereus*, *Bacillus laterosporus*, *Bacillus sphaericus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Enterobacter cloacae*, *Pseudomonas fluorescens*, *Pseudomonas stutzeri* and *Aeromonas hydrophila*. Meanwhile, there are 4 species of fungus that have been identified from the sampling sites, which are *Aspergillus niger*, *Aspergillus nidulans*, *Penicillium digitatum* and *Fusarium dimerum*. 
| Sampling Site | Bacteria Species       | Number of Colony/ m² / 24 h | Fungal Species          | Number of Colony/ m² / 24 h |
|---------------|------------------------|----------------------------|-------------------------|----------------------------|
| 1             | Micrococcus leteus     | 8                          | Aspergillus niger       | 5                          |
|               | Bacillus cereus        | 2                          | Penicillium chrysogenum | 5                          |
|               | Aeromonas hydrophila   | 18                         | Fusarium dimerum        | 18                         |
|               | Staphylococcus epidermis | 17                      |                         |                            |
| 2             | Micrococcus leteus     | 70                         | Aspergillus niger       | 3                          |
|               | Bacillus cereus        | 20                         | Penicillium chrysogenum | 8                          |
|               | Pseudomonas stutzeri   | 12                         | Fusarium dimerum        | 18                         |
|               | Staphylococcus epidermis | 40                     |                         |                            |
|               | Aeromonas hydrophila   | 7                          |                         |                            |
| 3             | Micrococcus leteus     | 75                         | Aspergillus niger       | 3                          |
|               | Staphylococcus epidermis | 110                    | Fusarium dimerum        | 5                          |
|               | Staphylococcus aureus  | 15                         |                         |                            |
|               | Enterobacter cloacae   | 12                         |                         |                            |
|               | Pseudomonas fluorescens | 3                        |                         |                            |
| 4             | Bacillus sphaericus    | 7                          | Aspergillus niger       | 12                         |
|               | Bacillus laterosporus  | 12                         | Penicillium chrysogenum | 5                          |
|               | Staphylococcus epidermis | 10                     | Fusarium dimerum        | 35                         |
| Sampling Site | Bacteria Species       | Number of Colony/ m² / 24 h | Fungal Species      | Number of Colony/ m² / 24 h |
|---------------|------------------------|----------------------------|---------------------|----------------------------|
|               | Staphylococcus aureus  | 3                          |                     |                            |
| 5             | Micrococcus leteus     | 7                          | Aspergillus niger   | 3                          |
|               | Staphylococcus epidermis | 35                      | Aspergillus nidulans | 7                          |
|               |                       |                            | Penicillium chrysogenum | 33                        |
|               |                       |                            | Fusarium dimerum    | 10                        |
| 6             | Micrococcus leteus     | 7                          | Aspergillus niger   | 17                        |
|               | Bacillus cereus        | 30                         | Penicillium chrysogenum | 38                        |
|               | Staphylococcus aureus  | 8                          | Fusarium dimerum    | 7                          |
|               | Aeromonas hydrophila   | 28                         |                     |                            |
| 7             | Micrococcus leteus     | 15                         | Fusarium dimerum    | 2                          |
|               | Bacillus cereus        | 3                          |                     |                            |
|               | Staphylococcus epidermis | 12                      |                     |                            |
|               | Pseudomonas stutzeri   | 2                          |                     |                            |
| 8             | Micrococcus leteus     | 8                          | Aspergillus niger   | 5                          |
|               | Bacillus sphaericus    | 5                          | Penicillium chrysogenum | 5                          |
|               | Bacillus cereus        | 6                          | Fusarium dimerum    | 2                          |
|               | Staphylococcus epidermis | 50                      |                     |                            |
|               | Pseudomonas stutzeri   | 3                          |                     |                            |
The entomology laboratory showed the greatest number of bacteria colonies in comparison to other laboratories, with a total of 215 bacteria colonies, while the Environmental Science Teaching Lab and Biology Building Main Lobby showed the lowest number of total bacteria colonies with only 32 colonies have been found for each site respectively.

In addition, the Environmental Microbiology Laboratory showed the highest number of fungus colonies, with a total of 62 colonies while the Biology Building Main Lobby showed the lowest number of fungus, with only just two colonies have been found. The difference in the number of fungus colony is possible due to the type of research done by the laboratory, the Environmental Microbes Laboratory is the place where researches on the various species of fungus have been conducted and this led to the increasing number of fungus colony. In addition, the difference in the number of fungus can also be driven by laboratory sanitary factors as well as the air-conditioning for each laboratory.

**The Measurement of CO$_2$, Temperature and Relative Humidity**

Table 2 shows the data of CO$_2$, temperature and relative humidity. All the sampling sites have recorded the CO$_2$ concentration below the standard that underlined by The Department of Safety and Health (DOSH) Malaysia which the CO$_2$ concentration for research lab cannot exceeding 1000 ppm (DOSH 2005). For the average temperature, the Environmental Science Teaching Laboratory has recorded the highest average temperature during daytime with 26.6 °c. This is due to the failure of its air conditioner to operate properly because of bad maintenance and increasing number of people that use the lab. For relative humidity, Biochemistry teaching lab has the highest percentages with 85.1% and the lowest percentages of relative humidity is Biology Building Main Lobby. Relative humidity basically relates with the size of area, which the wider the area the higher the relative humidity.
Table 2
The Measurement of CO₂, Temperature and Relative Humidity

| Sampling Site | Carbon dioxide (CO₂) (ppm) | Temperature (°C) | Relative Humidity (%) |
|---------------|-----------------------------|------------------|-----------------------|
|               | Day                        | Night            | Day                   | Night                  |
| 1             | 351 ± 5.8                  | 405 ± 12.1       | 23.0 ± 0.1            | 24.4 ± 0.3             | 85.1 ± 1.4 | 81.0 ± 0.5 |
| 2             | 473 ± 12.1                 | 435 ± 12.7       | 24.0 ± 0.5            | 25 ± 0.8               | 63.1 ± 2.4 | 67.7 ± 5.2 |
| 3             | 404 ± 30.2                 | 412 ± 22.5       | 23.0 ± 0.5            | 26 ± 1.1               | 63.4 ± 3.4 | 63.7 ± 4.4 |
| 4             | 412 ± 31.1                 | 466 ± 41.5       | 26.2 ± 0.7            | 27 ± 0.8               | 67.1 ± 2.0 | 69.5 ± 8.0 |
| 5             | 399 ± 20.0                 | 445 ± 17.9       | 24.6 ± 0.7            | 24 ± 0.5               | 77.7 ± 3.6 | 81.6 ± 5.2 |
| 6             | 442 ± 24.0                 | 403 ± 7.6        | 23.2 ± 0.4            | 24 ± 0.4               | 76.7 ± 1.7 | 78.0 ± 1.5 |
| 7             | 406 ± 84.3                 | 408 ± 13.7       | 24.0 ± 0.7            | 25 ± 0.7               | 49.0 ± 2.6 | 61.3 ± 3.0 |
| 8             | 402 ± 18.5                 | 402 ± 13.6       | 26.0 ± 0.6            | 25 ± 0.4               | 68.5 ± 3.1 | 66.2 ± 6.9 |
| DOSH          | 1000                       |                  | 23.0–26.0             | 40.0–70.0              |
| Standard      | 1000                       |                  | 22.5–25.5             | ≤ 70                   |
| SIAQC         | 1000                       |                  | 22.0–24.0             | 40–60                  |
| ASHRAE        |                            |                  |                       |                       |

The Level of User Satisfaction

Based on the survey conducted on 32 respondents, most of the respondents (16%) were only aware of the degradation of indoor air quality when there is an unpleasant smell. In addition, there are a number of respondents (34%) whom are not aware that there are several pollutants produced at indoor environment that can affect their health. However, there are some respondents (44%) that has a good level of awareness on indoor air quality on the importance of the ventilation system in a building as well as the impact of the internal air quality deterioration of the health of the person on the building. In addition, most of the respondents (81%) urge for a good ventilation system to improve indoor air quality in their workplace.

Discussion

The bacteria that have been found such as Micrococcus sp., Bacillus sp. and Staphylococcus sp. and fungus such as Aspergillus sp., Penicillium sp. and Fusarium sp. are common at indoor building. According to Giulio et al. (2010), who did a research at Faculty of Pharmacy, University of Chieti, Italy, the study also found the same species at almost all sampling sites. Micrococcus sp. is categorized as the normal flora of the human skin. The infection of Micrococcus luteus will lead to chronic skin diseases especially to the people with weak immune system (Smith et al. 1999; Azad et al. 2014). Staphyloccocus
*aureus* can cause the skin disease, as well as contagious disease such as bacteremia, food poisoning and also toxic shock syndrome due to toxin secretion (Jarraud et al. 2002). Meanwhile, for *Staphylococcus epidermis*, this species can infect human respiratory system which could cause lung inflammation and lung nosocomial pneumonia (Raad et al. 1998; Fu et al. 2020).

The dispersion of tiny, light and small spores by *Aspergillus* sp. and *Penicillium* sp. make it exists in the surrounding longer than the heavier and bigger spores (Vonberg & Gastmeier, 2006), and this type of spores can cause the asthma to human (Pastuszka et al., 2000; Prabhakaran et al. 2016). Health problem that cause by fungus is usually due to is mycotoxin. Mycotoxin is the secondary metabolites that produce by fungus that can cause serious illness to human such as degradation of immune system, nerves system failure, and organ dysfunction (Johanning et al. 1996; Khamal et al. 2019).

The other environmental factors such CO₂, temperature and relative humidity did not show any abnormal findings, but in certain sites, the condition could get worse. According to Black (1996), poor environmental condition would encourage the growth of bacteria and fungus which will lead to indoor microbiological air contamination. Besides that, these factors play a vital role towards human happiness in the building where good environmental factors will increase the mood and emotion, and will also increase human productivity (Fang et al. 2004)

**Conclusion**

Overall, this study has found 660 colonies of bacteria and 47 colonies of fungus from all sampling sites with 9 species of bacteria and 4 species of fungus. All type of species from both bacteria and fungus are known as the normal flora to human. But, excessive number of these microbes may cause illness to the human. This study also found that the environmental factors condition in the building are in a good condition. But, the awareness of users towards indoor air contamination remain low, while the satisfaction is high among users. Serious effort needs to be taken in maintaining the ventilation system of the building to avoid serious indoor microbiological air contamination. It is also recommended that users are suggested not to eat and drink inside the building, and avoid to stay in the building overnight.

**Declarations**

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable
Availability of data and material

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

Competing interests

The authors declare that they have no competing interests

Funding

This research has been supported by Universiti Kebangsaan Malaysia through research grant (GUP-2018-032)

Authors' contributions

SMM carried out the field work and lab studies, also write the first draft of the article, AH provides statistical analysis data, WSA is the lab supervisor and AA provide the proofreading and wrote the final draft.

Acknowledgements

Not applicable

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