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INDOOR MICROBIAL CONTAMINATION AND ITS RELATION TO PHYSICAL INDOOR AIR QUALITY (IAQ) CHARACTERISTICS AT DIFFERENT LABORATORY CONDITIONS

Hazrin, A. H.[a*], Anis Syazana, A. A.[a], Noor Faizul Hadry, N.[a], Norhidayah, A.[b], Mohd Shukri, M. A.[a]

[a]Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Kuantan Campus, Pahang, Malaysia
[b]Department of Occupational Safety and Health, Faculty of Technology, University Malaysia Pahang, Gambang Campus, Pahang, Malaysia

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*Corresponding authors
hazrinhadi@gmail.com

Abstract

Laboratory usually refers to a room or building equipped with chemicals and biological agents for scientific experimentation and research. Due to its own indoor hazards and its cause of health implications, it is an urge to assess and to understand the physical indoor air quality (IAQ) characteristics in the laboratory and the variables affecting the degree of exposure to occupants. The main objectives of this study was aimed to assess and to compare the physical IAQ characteristics and airborne pollutants including particulate matters (PM) and gaseous pollutants between laboratories, to identify microbial contaminants via bacterial counts as well as scientific bacterial-kits species identification and to correlate the relationship of physical IAQ characteristics, airborne pollutants and microbial contaminants between different laboratory settings within the same building which are Natural Product (NP) laboratory, Plant Tissue Culture (PTC) laboratory, and Microbiology laboratory. The physical IAQ characteristics and airborne PM was measured using VelociCalc multi-function ventilation meter 9565 and DustMate environmental dust detector respectively. Surface Air System Indoor Air Quality (SAS IAQ) was used to capture the microbial contaminants and after that bacterial counting and identification were done. The scientific method protocol and standard reference limits were compared based on Industrial Code of Practice on Indoor Air Quality (ICOP) (2010) regulated by the Department of Occupational Safety and Health (DOSH). The temperature of PTC laboratory, velocity of NP laboratory and Microbiology laboratory, the respirable particulate matter (PM) of all three laboratories and Colony Forming Unit (CFU) count of PTC and NP laboratory exceeded the standard limit regulated by DOSH. This study demonstrated that *Gemella morbillorum* is the common bacterial species available in the environment with poor IAQ and there is a significant relationship between physical characteristics, airborne pollutants and microbial contaminants between the different types of laboratory settings. In conclusion, priority should be given to NP laboratory as it is exposed to poor IAQ conditions and immediate action should be taken to eliminate the problems.

Keywords: Indoor air quality (IAQ), laboratory, airborne pollutants, particulate matter (PM), microbes, CFU

1.0 INTRODUCTION

Laboratory usually can be referred as a space room or building equipped for scientific research or teaching purposes including practicing, observing, and testing of chemicals, drugs, and other biological materials. A clean and optimum indoor environment of the laboratory is crucial to ensure the well-being of the occupants and also to keep all the expensive instruments, materials, and samples in a good condition [1]. To provide such hygienic, safe, and appropriate environment of work, any laboratory should comply with the requirement of standard guidelines provided by Department of Occupational Safety and Health (DOSH) under Industry Code of Practice on Indoor Air Quality (ICOP) (2010) [2].

To create a desirable environment, most enclosed building including laboratory often designed and

supplied with a mechanical ventilation air conditioning (MVAC) system for the purposes of airborne contaminants removal and to regulate the temperature, relative humidity (RH), and its ventilation rate [3]. However, a concern has been raised regarding the issue of poor maintenance and services of air ventilation system which could be a major factor that contribute to the increase in level of air pollutants through various kinds of mechanisms [1], [4] as well as a breeding ground for harmful bacteria which are then dispersed through the ventilation process [5]. Other than that, lapses in laboratory operating protocol and several kinds of human activities within the premises also may contribute to poor IAQ [4]. Poor IAQ of laboratory indoor environment have been known to cause a wide range of adverse effects towards the occupants due to exposure towards numerous hazardous airborne pollutants [6]; including Sick Building syndrome (SBS) [5], [7] and Building Related Illnesses (BRI) [8].

As some researchers and staff often spend hours working in the laboratories and currently limited research on IAQ of laboratory settings, it is necessary to assess the condition and characterize the airborne pollutants found in the laboratories and identifies the possible factors that may associate with the IAQ level for the safety and health concerns of the occupants.

2.0 MATERIALS AND METHODS

2.1 Sampling Site and Description

Walkthrough investigation was conducted to determine the laboratories characteristics and sampling sites. The three laboratories selected for IAQ assessment were NP Laboratory, PTC laboratory, and Microbiology laboratory. Based on their distinct characteristics in terms of location, activity, and function, these laboratories were assumed to represent the laboratory environments in Kulliyyah of Science, International Islamic University Malaysia (IIUM) Kuantan campus, Malaysia. Table 1 shows the details of the selected sampling sites of the study:

Table 1 Summary of walkthrough investigation

Laboratory	Sampling point	Occupants	Main activities
NP Lab.	2 (Work bench and officer's room)	5*	Testing of food samples and extraction of natural products.
PTC Lab.	2 (Work bench and preparation room)	4*	Culturing parts of plants including plant tissue.
Microbe. Lab.	1 (Work bench)	3*	Culturing and incubation of microbes.

*average occupants working in the laboratories

2.2 Instrumentation and Sampling Protocol

2.2.1 PM and Physical IAQ Assessment

PM and physical IAQ assessment was conducted by using two main instruments; DustMate environmental dust detector by Turnkey Instruments and VelociCalc multi-function ventilation meter 9565 by TSI®. DustMate environmental dust detector was used to measure the concentration of PM in four different fractions; thoracic, inhalable, respirable, and ultrafine particles (μg/m^3). VelociCalc multi-function ventilation meter 9565 was used to measure the level of physical IAQ including air velocity, air flow, relative humidity (RH), temperature, and carbon dioxide (CO_2). The method for air monitoring chosen was real time sampling with duplication of samples for each sampling points.

2.2.2 Airborne Microbial Assessment

SAS IAQ Microbial Air Sampler by PBI International was used to evaluate the microbial air contamination of the laboratory. The instrument was used together with agar plate (Nutrient Agar (NA) and Luria Bertani (LB)); which the surrounding air was aspirated over the agar surface of the plate and airborne particles were captured on the agar by impaction. Sampling duration was set to be 60 seconds with flow rate of 15 litres of air per minute. After sampling, the agar plate was removed and incubated in the laboratory for 16 hours at 37°C. After that, colony forming unit (CFU) was counted.

2.3 Laboratory Analysis

2.3.1 Morphological Identification

After the plates incubated, colonies of the microbial organisms were counted and recorded by using the following formula:

1 minute sample at 15L/min = 15 L of air samples
15L = 15/1000 = 0.015m^3
CFU N = N/0.015m^3 = ____ CFU/m^3

2.3.2 Pure Culture

Twelve colonies of bacteria were chosen from each sample by using sterilized inoculating loop and placed in twelve respective grids on the new plate. The bacteria were then incubated for 16 hours at 30°C. After those colonies with different colours or morphology chosen, it will be streaked on the new plates for identification tests. The plates then will be incubated again for 16 hours at 30°C.

2.3.3 Cell Morphology

Cell morphology was determined by using Gram staining method. The slides were observed by using Dino Eye Equipment. Bacteria were characterized by their Gram staining, colour, shape, and arrangement. Classification of bacteria was done according to their colony and cell morphology.

2.3.4 Bacterial Identification Using API System

Analytic Profile Index (API) strips were used for bacterial colony identification. API 20E was used for identification of Enterobacteriacae and other non-fastidious Gram negative bacteria. Complementary tests including oxidase test, oxidation fermentation test, motility test, and MacConkey test were complementary to be conducted upon identifying the bacteria. API 20 Strep was used to identify Gram positive bacteria. It was used to identify most streptococci. The complementary test for API 20 Strep includes the blood agar test.

2.3 Data Analysis

The data of the parameters were analysed by using Kruskal-Wallis test, a statistical hypothesis test to determine the statistical differences between two or more groups of an independent variable. A p-value less than 0.05 were considered statistically significant.

3.0 RESULTS AND DISCUSSION

3.1 Assessment of IAQ Parameters

The data obtained from the studies are presented in daily average and compared to the standard established by DOSH as in 8-hour exposure of time as in Table 2.

Table 2 Results of daily average of physical IAQ parameters and airborne pollutants between laboratories and comparison with standard reference ICOP (2010)

	Parameters	*Acceptable Limit by DOSH*	*Locations*			*P value*
			PTC laboratory	NP laboratory	Microbiology laboratory	
Physical IAQ	Temperature (°C)	23 – 26	**22.18 ± 1.71**	23.32 ± 0.61	24.60 ± 0.79	0.041*
	RH (%)	40 – 70	64.41 ± 4.38	53.89 ± 3.36	59.83 ± 5.06	0.008*
	Air velocity (m/s)	0.15 – 0.50	0.19 ± 0.05	**0.08 ± 0.07**	**0.12 ± 0.05**	0.035*
	Air flow (m^3/h)	NA	21.92 ± 5.51	9.46 ± 8.12	13.60 ± 5.42	0.042*
	CO_2 (ppm)	1000	476.50 ± 64.74	608.17 ± 80.03	461.67 ± 13.65	0.020*
Airborne Pollutants	Inhalable (mg/m^3)	NA	1.76 ± 1.38	3.94 ± 4.21	2.90 ± 3.03	0.625
	Thoracic (mg/m^3)	NA	1.13 ± 0.96	1.92 ± 1.70	1.39 ± 0.77	0.769
	Respirable (mg/m^3)	0.15	**0.64 ± 0.17**	**0.65 ± 0.42**	**0.45 ± 0.31**	0.864
	Ultrafine (mg/m^3)	NA	0.37 ± 0.40	0.30 ± 0.26	0.19 ± 0.13	0.988
	CFU (CFU/m^3)	500	**1145.00 ± 8.50 x 10^2**	**2856.00 ± 2.65 x 10^3**	422.33 ± 1.68 x 10^2	0.079*

1. *significant at p<0.05
2. Bold readings are the values that exceed the acceptable limit set by DOSH

3.1.1 Physical IAQ Parameters

From the results in Table 2, most of the physical IAQ parameters measured were within the range of acceptable limit recommended by ICOP (2010) except for air velocity as poor condition were noticed in the NP and Microbiology laboratory during the assessment. Other than that, the temperature was found slightly lower than the recommended setting in the PTC laboratory. These unfavourable conditions including poor air ventilation, temperature, and relative humidity may contribute to the increase of some indoor pollutants [9].

The air ventilation is very important for the indoor environment setting; either supplied with mechanical or natural ventilation system as inadequate air exchange may favour the accumulation of pollutants inside, combined with other additional indoor sources [9]. The low air velocity and air flow commonly associated with poor air exchange rate of the premises and indicated by the high level of CO_2 as its concentration is a key parameter for assessing IAQ and the building ventilation efficiency and indicator of inadequate air exchange [10], [11]. The facts also supported by the finding of a study on IAQ in three different buildings which found CO_2 concentration represented the ventilation performance of building, thus the building with higher CO_2 was subjected to the poor ventilated indoor air due to imbalance in the ventilation system, allowing the accumulation of possible contaminants with the indoor environment [12]. As a suggestion, the poor ventilation system and air exchange efficiency of the laboratory can be confirmed by conducting gas concentration decay test.

Other than that, the CO_2 concentration in the laboratory also might be contributed by the occupants. The previous studies on IAQ in the laboratories mentioned that human are the main sources of CO_2 within the air-conditioned spaces due to respiration activities [1] and the elevation of the concentration was in concordance with the number of occupants presence in the premise [13]. Even if the laboratory could accommodate large number of occupants, the poor ventilation system of the laboratory could lead to accumulation of CO_2 in the premise over time. Although such level poses no health problems, it should be resolved as soon as possible to avoid any adverse health problems.

3.1.2 Airborne Pollutants

For the assessment of airborne pollutants, it was found that the concentration of respirable particles in all of the selected laboratories exceeded the acceptable limit set by DOSH in ICOP (2010). Overall, NP laboratory has recorded the highest concentration of airborne pollutants compared to others except for ultrafine particles. Since this laboratory showed the greatest level of most IAQ parameters as well, this proved that airborne pollutants level very much related to physical IAQ, which later affects the bacterial CFU count.

In indoor environment, the sources of PM can come from both indoor and outdoor sources which are affected by numerous kinds of factors such as air exchange rates, ambient concentrations, penetration factors, and also mechanisms of deposition and resuspension of PM [14]. During the investigation, the ventilation system found to be insufficient to ventilate the contaminants to the outside. Issue of chemical storage inside the lab without proper ventilation may contribute to the findings exceeding the acceptable limit. It was observed that the MVAC system within the area was centralized, where it could be concluded that the potential exposure in the office might due to potential of cross contamination from the teaching area and the preparation room.

In addition, the great amount of airborne total particle mass concentrations normally occurred in spaces with high occupancy. This result was consistent with the data of occupancy being the most dominant source of particulate matter indoor [15]. Moreover, the effects of number of occupants and their activities in the indoor environment towards the concentration of PM had been seen and proved in previous studies on IAQ [11], [15] and it might be strongly due to the import of particles or by the resuspension of previously deposited particles or dusts through their movements and activities [11], [17]. As the data for airborne pollutants in concordance with the data from physical IAQ assessment, it can be confirmed that occupancy played a significant role in affecting the quality of the indoor air.

3.1.2 CFU Counting

NP laboratory reached the highest number of total bacteria. A higher number of total bacteria could be associated with the high percentage of relative humidity as bacteria grow rapidly on moist and condensed area. Moreover, higher humidity could cause the pathogenic bacteria to attach to droplets discharged into the air, thus causing infections, which brought to respiratory diseases [18]. This data was not in concurrence to the findings collected in university classrooms where the presence of microorganisms in air was the highest during simple ventilation and when the air conditioning was activated [19].

However, it was explained that this may be due to the poor ventilation system and inadequate maintenance of the MVAC system or the use of water-damaged carpet as some of the various factors that could result in high bacterial counts [5] and due to contaminated air handling system which could become a breeding zone grounds for biological contaminants [18]. Furthermore, bacterial in indoor environment may also come from wet or moist walls, ceiling, carpets, poorly maintained humidifiers, and air conditioned [20]. It was also mentioned that bacterial counts in indoor environment were commonly higher in crowded

places and did not necessarily emphasize that human infections would occur but served as screening tests for further investigation [5].

3.2 Bacterial Identification using API System

Table 3 Bacterial identification from API Web

Strain	Total groups of colony	Percentage (%)	Note	Gram (+/-)	PTC Laboratory	NP laboratory	Microbiology Laboratory
Listeria spp	12	87.7	Acceptable identification	+	√	√	
Gemella morbillorum	15	94.9	Good identification	+	√	√	√
Gemella morbillorum	19	69.9	Low discrimination	+	√	√	√
Gemella morbillorum	29	56.0	Low discrimination	+	√	√	√
Lactococcus lactis spp lactis	13	81.8	Low discrimination	+	√		
Leuconostoc spp	4	99.7	Very good identification	+	√		
Gemella morbillorum	10	69.9	Low discrimination	+		√	
Gemella morbillorum	7	69.9	Low discrimination	+		√	√

Based on the results in Table 3, *Gemella morbillorum* was the most prevalent bacteria found in the laboratories. *Gemella morbillorum* is a Gram positive coccus which usually found in the normal flora of human mucous membrane. This bacterium commonly associated to endocarditis though its occurrence caused by it is very rare [21]. Since it is a normal inhabitant of oral cavity, its presence in the laboratory environment might be originated from the occupants. In addition, it can be assumed as a common bacterium in indoor environment as similar bacterium also found in another research conducted in enclosed building [22]. Another species found were *Listeria spp., Leuconostoc spp., and Lactococcus lactis.* However, they were not presence in all laboratories. Listeria genus can generally be isolated from various food products including dairy products, meat, vegetables, and seafood and act as a potential risk when raw partially processed and even some fermented food are consumed. This bacterium commonly associated with food contamination which resulted in listeriosis. It is widespread in the environment especially in the air hence usually collected in environmental samples as well [23]. In addition, this explained why this bacterium was collected in PTC laboratory and NP laboratory only.

The other bacterium is *Leuconostoc spp.*, generally a non-pathogenic acid-tolerant organism with optimal temperature of 18°C to 25°C. It is commonly available in many different processed foods, either as a starter culture or as a contaminant. *Leuconostoc spp.* is a chemoorganoheterotrophic bacteria and a facultative anaerobe that are listed as environmentally related microbe. As *Leuconostoc spp.* predominantly reported and found in many plant materials, it can be assumed that its primary sources in the laboratory environment were coming from tissue culturing activities by the researchers [24]. Another species found is *Lactococcus lactis*, a non-pathogenic Gram-positive bacterium which usually occupies a niche related to plant or animal surfaces in nature. So, it can be assumed that its main sources in the laboratory were originated from plant materials used for experimentation activities [25]. *Leuconostoc spp.* and *Lactococcus lactis* bacteria were found in the PTC laboratory only.

4.0 CONCLUSION

In conclusion, there was a significant relationship between physical characteristics, airborne pollutants, and microbial contaminants between the different types of laboratory settings. The CFU counting was greater when the temperature was lower and CO_2 level was high which resulted in low relative humidity, air velocity and flow. The ventilation system also plays an important role in the increase of airborne pollutants and affecting the IAQ. In addition, the IAQ parameters and airborne pollutants were possibly associated with the level of occupancy and activities of the occupants. Preliminary actions should

be taken to ensure the IAQ of the laboratories in compliance with the standard provided by DOSH in Industry Code of Practice for Indoor Air Quality (ICOP) 2010 and to provide a safe and healthy workplace environment.

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