

The correlation of *Acanthamoeba* from the ventilation system with other environmental parameters in commercial buildings as possible indicator for indoor air quality

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Abstract: The free-living protozoan *Acanthamoeba* is an opportunistic pathogen that is ubiquitous in our environment. However, its role in affecting indoor air quality and ill-health of indoor occupants is relatively unknown. The present study investigated the presence of *Acanthamoeba* from the ventilation system and its correlation with other indoor air quality parameters, used in the industry code of practice and its potential as an indicator for indoor air quality. Indoor air quality assessments were carried out in nine commercial buildings with approval from the building management, and the parameters assessed were as recommended by the Department of Occupational Safety and Health. The presence of *Acanthamoeba* was determined through dust swabs from the ventilation system and indoor furniture. Logistic regression was performed to study the correlation between assessed parameters and occupants' complaints. A total of 107 sampling points were assessed and 40.2% of the supplying air diffuser and blowing fan and 15% of the furniture were positive for cysts. There was a significant correlation between *Acanthamoeba* detected from the ventilation system with ambient total fungus count ($r=0.327$; $p=0.01$) and respirable particulates ($r=0.276$; $p=0.01$). Occupants' sick building syndrome experience also correlated with the presence of *Acanthamoeba* in the ventilation system ($r=0.361$; $p=0.01$) and those detected on the furniture ($r=0.290$; $p=0.01$). Logistic regression showed that there was a five-fold probability of sick building syndrome among occupants when *Acanthamoeba* was detected in the ventilation system.

Key words: *Acanthamoeba*, Indoor air quality assessment, Opportunistic pathogen, Ventilation system

Practical Implications: The present study showed the significant association between the presence of *Acanthamoeba* and other air quality parameters, and sick building syndromes in the occupants. Indoor air quality assessment should therefore include the detection of *Acanthamoeba* cyst in addition to that of other traditional biological parameters.

Introduction

The indoor environment is designed to protect occupants from outdoor hazards. Commercial buildings and complexes with office space, multipurpose halls and hotels, are designed and regulated to provide optimum comfort and

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Table 1. The Locations of IAQ Assessment in the Present Study

Building Types	No. of sampling points	Type of MVAC	Use of Indoor Space	Remark
Academic Institution	57	Centralised AHU, Discrete Air Conditioning	Office Setting	Building A
Hotel	4	Centralised AHU	Office Setting, Canteen	Building B
Commercial Tower	4	Centralised AHU, Discrete Air Conditioning	Meeting Rooms	Building C
Commercial Tower	5	Centralised AHU	Office Setting	Building D
Commercial Tower	8	Centralised AHU	Office Setting	Building E
Commercial Tower	11	Centralised AHU	Office Setting	Building F
Manufacturing	4	Centralised AHU	Central Command Room	Building G
Manufacturing	2	Fan Coil Unit	Enclosed Production Room	Building H
Manufacturing	12	Centralised AHU, Discrete Air Conditioning	Enclosed Production Room	Building I

*Indicated sampling points do not include reference point used in the assessment such as outdoors air sampling. A minimum of 1 sampling point for every 500 m² MVAC serving area is observed.

healthy environment to the occupants, including adequate ventilation, thermal comfort and lighting. Any faulty or poorly maintained mechanical ventilation and air conditioning (MVAC) systems are known to predispose occupants to discomfort, sick building syndrome (SBS), or building related illnesses (BRI). As such, regular monitoring and maintenance of MVAC operation is essential. Standards and guidelines have been established to ensure good indoor air quality (IAQ) as required by the industrial code of practice (ICOP). The ICOP measures various environmental variables pertaining to thermal comfort issues and other common physical, chemical and biological pollutants.

However, as the indoor environment is subjected to dynamic changes both temporarily and spatially through usage, the characteristics of indoor pollutants can also change drastically. The interaction of building air circulation and redistribution of pollutants originating from both indoors and outdoors can make the type of hazards difficult to trace and remediate. Furthermore, the occupants can be the source of pollutants themselves. Thus, besides periodic revision of ICOP, research into other potential pollutants present in the indoor environment is crucial.

Most IAQ studies have concentrated on environmental chemicals that cause acute adverse health effect to indoor occupants^{1,2}). Microflora such as bacteria and fungi have frequently been studied as well^{3,4}). However, not much work has been carried out to study microorganisms such as algae and protozoa in the MVAC and HVAC (Heating, Ventilation and Air Conditioning). Previous studies have shown that biological contamination of the HVAC could pose severe adverse health effects to the building occupants^{5,6}). *Legionella* from HVAC has also been known to cause pneumonia^{7,8}). To complicate matters, *Legionella* is shown to have the ability of using *Acanthamoeba* as its host (Bouyer *et al.* 2007) and could be protected from the

normal process of the HVAC cleaning.

Thus, it is important to understand the nature of *Acanthamoeba* presence in the indoor environment, especially MVAC and its possible role in IAQ. *Acanthamoeba*, which comprise of ubiquitous free-living amoebae in the soil and water are recognized as opportunistic pathogens that can cause ulcerative acanthamoebic keratitis⁹) and granulomatous amoebic encephalitis¹⁰). A recent study of some indoor mechanical air conditioning system showed the presence of various *Acanthamoeba* species in the filtrated dust¹¹). These organisms could serve as potential hazard to indoor occupants if the system is not hygienically maintained as the contaminated dust particles can be blown into the indoor environment by the mechanical fan.

It is the aim of the present study to look into the presence of *Acanthamoeba* in the indoor environment and its potential role as an indicator for indoor air quality. This could contribute to the better understanding and comprehensive protocol in IAQ assessment to protect building occupants from continuous exposure to such hazards and create a healthier building environment.

Material and Methods

Commercial buildings and factories installed with MVAC were selected for the present study. All buildings are located within Klang Valley area. Approval for IAQ assessment was obtained from the owners prior to the assessment. The selection criteria for the building included complete data collection based on ICOP parameters and questionnaire survey. Buildings and offices selected in the present study are listed as below (Table 1):

Building walkthrough and air sampling

Building walkthrough provides one of the most valuable tools to identify air quality problems. A planned walk-

Table 2. Employed Sampling Instruments in Indoor Air Quality Assessment

Parameters	Equipment	Manufacturer
Temperature & Humidity	Thermo hygrometer; YESAIR Air Quality Monitor	Oregon Scientific; Critical Environment Technologies™
Air Movement	Kestrel 100 weather meter	Nielsen Kellerman
Carbon Monoxide (CO)	Aspirating Pump AP20 with Gas Detector Tube System Range: 1–50 ppm; YESAIR Air Quality Monitor	Kitagawa, Japan Product Code: 106SC; Critical Environment Technologies™
Carbon Dioxide (CO ₂)	Aspirating Pump AP20 with Gas Detector Tube System Range: 100–1,500 ppm; YESAIR Air Quality Monitor	Kitagawa, Japan Product Code: 126B; Critical Environment Technologies™
Formaldehyde (CH ₂ O)	Aspirating Pump AP20 with Gas Detector Tube System Range: 0.05–4 ppm; YESAIR Air Quality Monitor	Kitagawa, Japan Product Code: 171SC; Critical Environment Technologies™
Ozone (O ₃)	Aspirating Pump AP20 with Gas Detector Tube System Range: 0.05–4 ppm; YESAIR Air Quality Monitor	Kitagawa, Japan Product Code: 182U; Critical Environment Technologies™
Total Volatile Organic Compounds (TVOC)	TVOC detector; YESAIR Air Quality Monitor	ION Science Ltd. Serial Number: 09-01372; Critical Environment Technologies™
Respirable Dust	APEX Sampling Pump with Higgins-Dewell cyclone and 37 mm, 5 µm PVC membrane; YESDUST Particulates Sensor	Casella UK Serial Number: 0663065; Critical Environment Technologies™
Carbon Dioxide (CO ₂)	Total Resources and Environmental Monitoring System (TREMS)	SRAS Bhd. Telasia Symtonic K3311170VC

through was carried at each building to determine the sampling location. Notes were taken on spatial comfort based on occupant density, ventilation level, dirt and dust, general cleanliness and hygiene, as well as potential contaminant sources.

The rules of minimum sampling points required at each sampling location were followed as prescribed in the ICOP manual¹²⁾. Samples were collected during the working hours from 0800 to 1700 hrs. During field data collection, instruments and air monitors were positioned at the selected sampling locations to represent the primary workstation layout and work activities, within the breathing zone of 75 cm to 120 cm from the floor and 1.0 m away from office electrical instrument or appliances like photocopier and printer. Studied parameters and indoor contaminants as listed in the ICOP were measured and recorded by calibrated instruments (Table 2). The measured parameters include: air temperature, relative humidity, air movement, carbon dioxide, carbon monoxide, formaldehyde, ozone, respirable particulates, total volatile organic compounds, total bacteria counts, total fungal counts.

Questionnaires survey

The Department of Occupational Safety and Health indoor environmental quality survey and checklist¹²⁾ were used to obtain information on the building and MVAC system and the general condition of the working environment. Building occupants were notified of the upcoming

IAQ assessment and verbal consent was obtained for the questionnaire survey. Information on the working conditions and the worker's health status were obtained. Sick Building Syndrome (SBS) was determined as prescribed in the ICOP 2010 guidelines. The presence of SBS is determined if more than 20 percent of the exposed occupant have complaints or experience adverse acute health and/or discomfort effects that are linked to time spent in a particular sampling point.

Examination of acanthamoeba and other microbiological samples

Total bacteria counts and total fungi counts were conducted at the sampling points as other parameters were taken. Air impingent sampler was employed with 200 ml of air samples were collected at each sampling plate. Duplicate media plates (MEA and TSA) were incubated at 30°C for up to five days. Colony forming units were counted and recorded from time to time after 24 hours of incubation. Dust swabbing was carried on the indoor furniture within the breathing zone (height between 0.7 m to 1.2 m) and the ventilation system (air supply diffuser, air conditioning unit blower) closed to the sampling point. *Acanthamoeba* cyst was examined for under the inverted light microscope. Two sampling containers were used for the dust swab, non-nutrient agar plate and 25 cm² tissue culture flask. Non-nutrient agar was then added with *E. coli* as food sources for viable *Acanthamoeba*. Culture flask was filled with

Page's saline. All samples were incubated at room temperature and observed daily for up to 14 days.

Statistical analysis

Statistical software SPSS™ (version 18) was employed in analyzing the parametric and non-parametric data. Univariate analyses were then performed between the variables; chi-square, Pearson's correlation and Spearman's non-parametric correlations were carried out as appropriate. The relationship between variables were found and significant level was set at $\alpha=0.05$.

The analysis was then carried out to determine the interaction of different variables at various levels. Logistic analysis was adopted in the present analysis to quantify the interaction effect among variables for the interested response. The presence of SBS at each sampling point was set as the dependent variables and the presence of *Acanthamoeba* in the ventilation as well as indoor environment were taken as possible contributing factors. The model was written in terms of a probability. Given the regressors x , then the logistic function is given by:

$$p = \frac{1}{1 + e^{-x\beta}}$$

Where p = probability of event
 $x\beta$ = Linear predictor

Ethical approval

This project was approved by the International Medical University Joint Committee on Research and Ethics (IMU-JC), Ref : 4.4/JC13/208.

Results

A total of nine buildings were assessed in the present study for the IAQ assessment and 107 sampling points within the occupant's activity areas were identified. A majority of the sampling points were served by centralized AHU with connecting duct while only 14 out of 107 sampling points are served with split fan coil unit (FCU). Indoor air temperatures recorded were relatively low (22.8°C) in areas served by the AHU compared to those served by FCU (25.3°C). Most of the assessed spaces were offices with moderate occupant activity like walking and paper documentation (Fig. 1).

Air sampling parameters

Generally, the mean temperature recorded was between the acceptable ranges of 23°C–26°C as recommended by the ICOP (DOSH, 2010). The RH recorded was also within

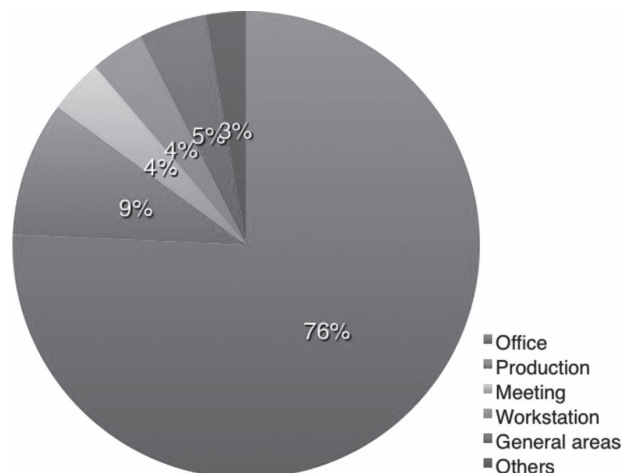


Fig. 1. The distribution of space used in the present IAQ assessment.

the 40%–70% acceptable range. Ozone was not detected across all sampling points. Most parameter means were within the ICOP except for respirable dust particulate and formaldehyde level. The details of recorded parameters are as shown in Fig. 2–Fig. 10.

Symptoms of SBS obtained from questionnaire survey

From 107 sampling points, 64 points (59.8%) were observed with reported occupant experience of sick building syndrome (Table 3). No building related illnesses were reported from the present study.

Sampling of acanthamoeba cyst

Swab sampling of dust showed only 40% of all sampling points to be positive for *Acanthamoeba* cyst in the supplying air diffuser and fan while 15% of furniture dusts were positive for cyst from the selected sampling points (Table 4).

Types of MVAC and IAQ

Although individual FCU comprised only 13% of the total serving MVAC units in the present assessment, there was a significant higher pollutant level associated with them when compared to centralized AHU. These parameters include respirable particulates, CO₂ and total volatile organic compounds (Table 5).

Correlation of SBS and IAQ

Statistical analyses on bivariate correlations using Pearson's correlation and Spearman's non-parametric correlations among IAQ parameters and SBS were determined. Significant correlation was seen between total bacteria

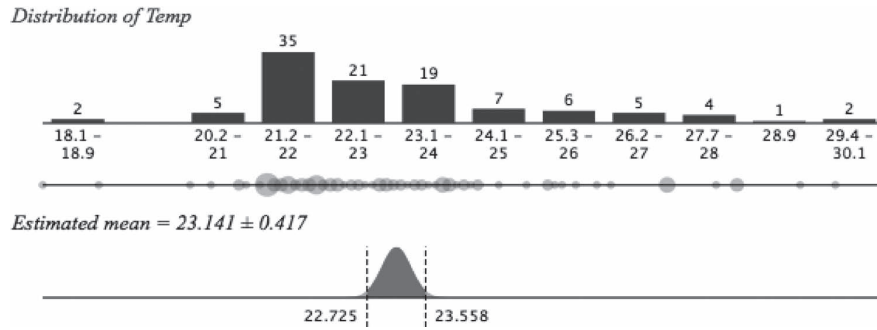


Fig. 2. Distribution of indoor temperature (°C) recorded in the present IAQ assessment; ICOP range: 23°C–26°C.

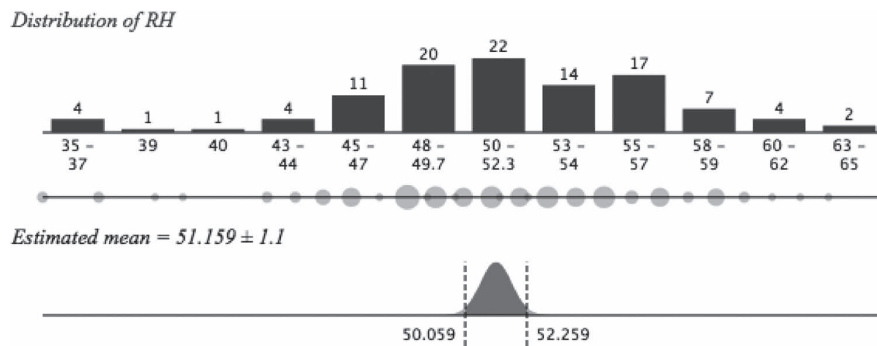


Fig. 3. Distribution of indoor relative humidity, RH (%) recorded in the present IAQ assessment; ICOP range: 40%–70%.

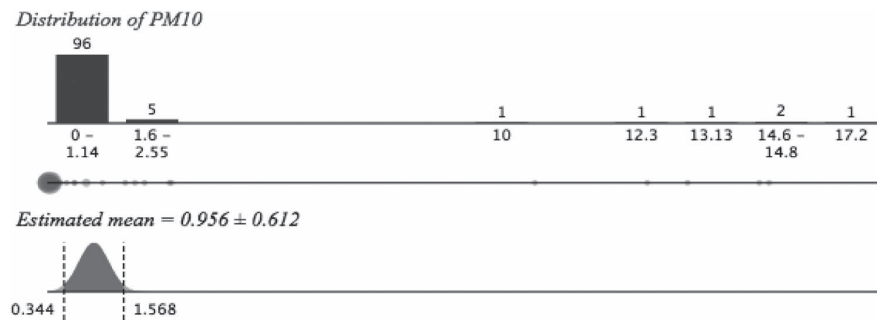


Fig. 4. Distribution of indoor respirable particulates, PM₁₀ (mg/m³) recorded in the present IAQ assessment; ICOP limit: 0.15 mg/m³.

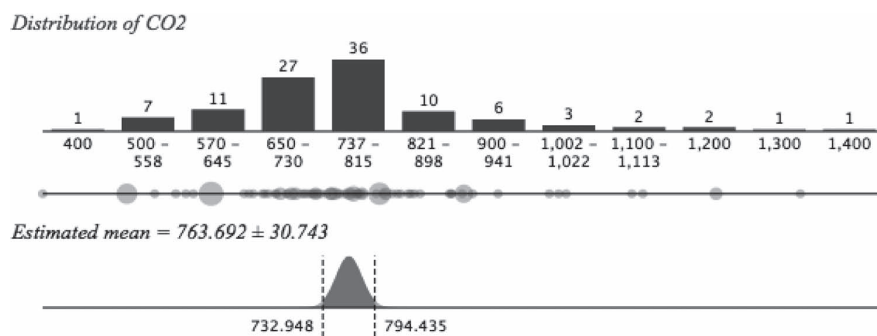


Fig. 5. Distribution of indoor carbon dioxide, CO₂ (ppm) recorded in the present IAQ assessment; ICOP ceiling limit: 1,000 ppm.

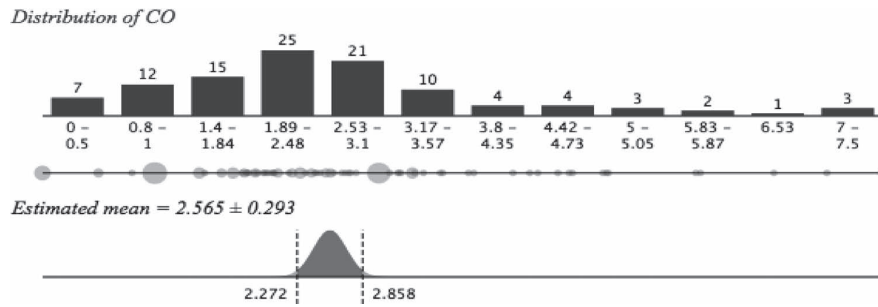


Fig. 6. Distribution of indoor carbon monoxide, CO (ppm) recorded in the present IAQ assessment; ICOP limit: 10 ppm.

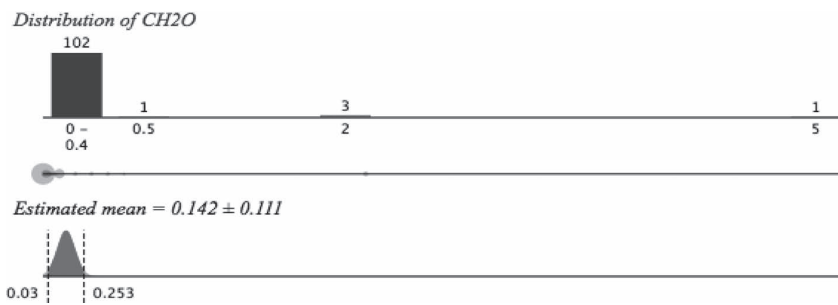


Fig. 7. Distribution of indoor formaldehyde, CH₂O (ppm) recorded in the present IAQ assessment; ICOP limit: 0.1 ppm.

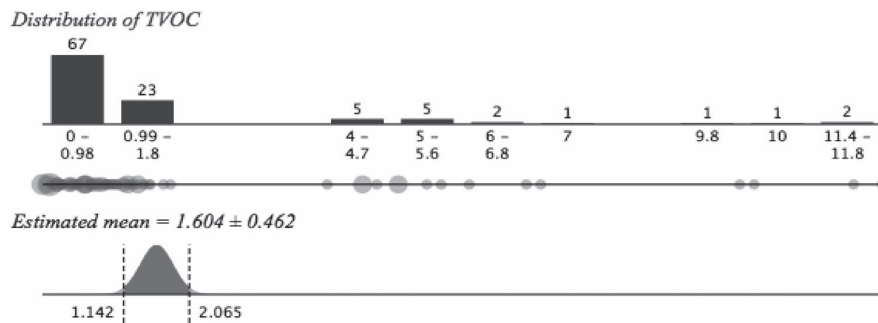


Fig. 8. Distribution of indoor total volatile organic compounds, TVOC (ppm) recorded in the present IAQ assessment; ICOP limit: 3 ppm.

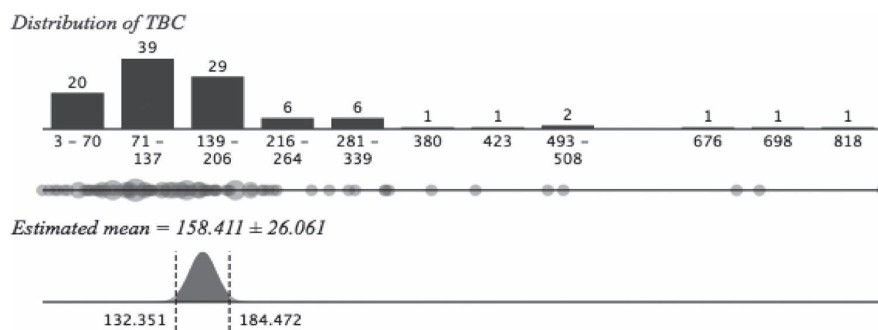


Fig. 9. Distribution of indoor total bacteria count, TBC (cfu/m³) recorded in the present IAQ assessment; ICOP limit: 500 cfu/m³.

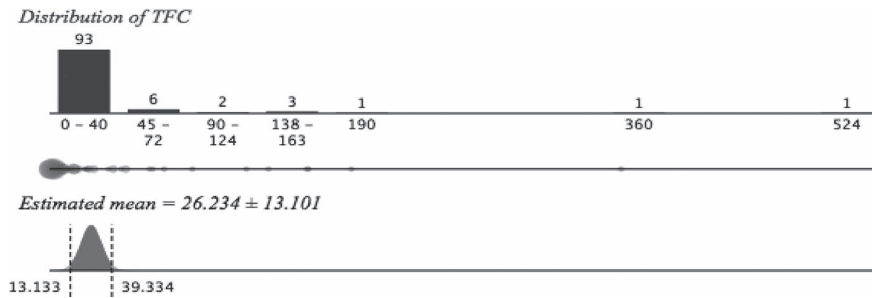


Fig. 10. Distribution of indoor total fungi count, TFC (cfu/m³) recorded in the present IAQ assessment; ICOP limit: 1,000 ppm.

Table 3. SBS symptoms observed among occupants

Location	SBS Observed among occupants (%)	List of most common symptoms
Building A	29/61 (47)	1. Irritated, stuffy nose 2. Hoarse, dry throat 3. Headache 4. Fatigue/Lethargy
Building B	22/29 (75)	1. Headache 2. Irritated, stuffy nose 3. Hoarse, dry throat
Building C	6/30 (20)	1. Irritation of eyes 2. Headache 3. Hoarse, dry throat
Building D	6/26 (23)	1. Hoarse, dry throat 2. Irritation of eyes
Building E	45/56 (80)	1. Irritation of eyes 2. Skin rash/itchiness 3. Hoarse, dry throat 4. Irritated, stuffy nose 5. Headache
Building F	6/30 (20)	1. Irritated, stuffy nose
Building G	5/15 (34)	1. Cough 2. Irritated, Stuffy Nose 3. Headache
Building H	9/24 (38)	1. Skin rash/itchiness 2. Hoarse, dry throat
Building I	16/31 (52)	1. Tired or stained eyes 2. Dry or itchy skin
Total	144/302	

Table 4. Sampling of *Acanthamoeba* from ventilation blowing diffuser and furniture dust

	Cyst from Blowing Vent		Cyst from Furniture Dust	
	Sampling Point	Percentage (%)	Sampling Point	Percentage (%)
Absent	64	59.8	91	85
Present	43	40.2	16	15
Total	107	100	107	100

count and total fungi count ($r=0.529$; $p=0.01$); total fungi count and *Acanthamoeba* detection in the ventilation ($r=0.327$; $p=0.01$); respirable particulates and *Acanthamoeba* in the ventilation ($r=0.276$; $p=0.01$); sick building

syndrome and presence of *Acanthamoeba* in the furniture dust ($r=0.290$; $p=0.01$); and sick building syndrome and *Acanthamoeba* in the ventilation ($r=0.361$; $p=0.01$ level).

There was no significant correlation between total bacteria count and *Acanthamoeba* cyst in the furniture dust ($r=-0.087$; $p>0.05$); total fungi count and *Acanthamoeba* in the furniture dust ($r=0.177$; $p>0.05$); total bacteria count and *Acanthamoeba* from ventilation ($r=0.113$; $p>0.05$); respirable particulates and *Acanthamoeba* in furniture dust ($r=0.186$; $p=0.06$); and *Acanthamoeba* in furniture dust and those from ventilation ($r=0.137$; $p=0.158$).

Logistic regression

The SBS logistic model with detection of *Acanthamoeba* in the ventilation and furniture in the present study can be calculated as shown (Fig. 11):

- Outcome variable: Sick Building Syndrome
- Explanatory variables: i. *Acanthamoeba* in the Ventilation
ii. *Acanthamoeba* on the furniture

$$SBS = 1 / \{1 + \exp[0.407 + 1.621(VantAca) + 2.48(FurAcan)]\}$$

Where, SBS = Probability of sick building syndrome to be observed at sampling point

VantAca = 0 with absence of *Acanthamoeba* in the Ventilation

1 with presence of *Acanthamoeba* in the Ventilation

FurAca = 0 when absent of *Acanthamoeba* on the furniture

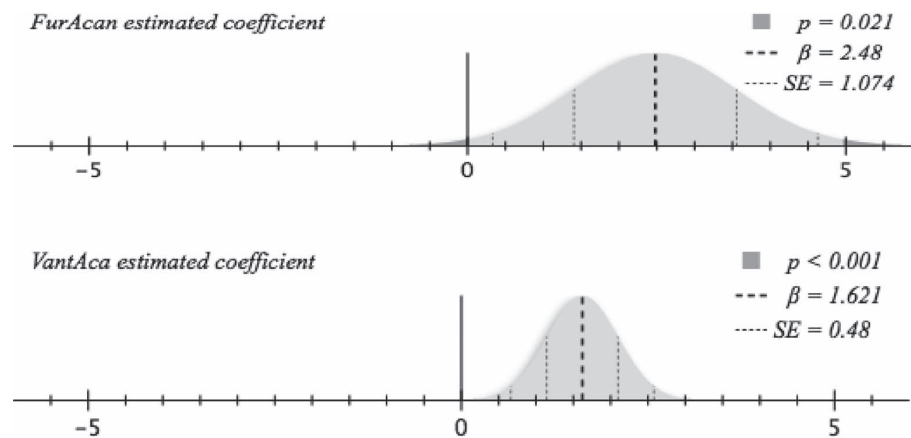
1 when present of *Acanthamoeba* on the furniture

Exp (B) VantAca = 5.060

Exp (B) FurAcan = 11.946

Table 5. Types of MVAC and related IAQ parameters in the present study

	Building		Commercial		Factory		Hotel	
			AHU	FCU	AHU	FCU	AHU	FCU
MVAC			AHU	FCU	AHU	FCU	AHU	FCU
Count			77	8	14	4	2	2
Temperature Mean (°C)			22.3	24.7	25.4	25.6	23.6	27
Relative Humidity; Mean (%)			52.6	41.9	46	51.5	62.5	56
Respirable Particulate; Mean (mg/m ³)			0.09	5.94	2.52	0.012	0.40	5.90
Carbon Dioxide; Mean (ppm)			757	725	764	938	600	975
Carbon Monoxide; Mean (ppm)			2.8	0.9	2.3	0.95	3	5
Formaldehyde; Mean (ppm)			0.014	0.212	0.857	0.025	0.050	0.100
Ozone; Mean (ppm)			>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
Total Volatile Organic Compounds; Mean (ppm)			0.744	3.150	4.936	4.550	0.250	0.650
Total Bacteria Count; Mean (CFU/m ³)			135	402	183	59	190	80
Total Fungus Count; Mean (CFU/m ³)			10	101	81	11	4	4
Detection of <i>Acanthamoeba</i> on Furniture Dust; Count (%)			9 (11.7)	0 (0)	2 (14.3)	2 (50)	1 (50)	2 (100)
Detection of <i>Acanthamoeba</i> on Ventilation Diffuser; Count (%)			19 (24.7)	6 (75)	14 (100)	1 (25)	1 (50)	2 (100)
Sick Building Syndrome; Count (%)			46 (59.7)	1 (12.5)	12 (85.7)	2 (50)	1 (50)	2 (100)

**Fig. 11.** Analysis of logistic regression on *Acanthamoeba* variables.

Discussion

The present study provides an overview of the IAQ scenario in tropical commercial buildings. Although the majority of the sampling points were in the office setting, the concentration of air pollutants varied depending on indoor environment and space setting.

IAQ parameters

Although the physical parameters like temperature and relative humidity readings are generally within the acceptable range of ICOP recommendations, temperature variances do occur throughout the day. This is especially significant in buildings installed with many glass panels. Fluctuations of 1°C to 2°C were normally observed from morning to afternoon. Likewise, the building section facing the sun would give a higher indoor environment tem-

perature, resulting in temperature variance across the floor throughout the day. Rainfall pattern was observed to affect the relative humidity in buildings, as most MVAC in the present study do not have any dehumidifier installed.

Respirable particulates or PM₁₀ measured in the present study contributed significantly to symptoms experienced by occupants. Previous studies have shown that the indoor PM₁₀ is positively significantly correlated with the presence of asthmatic symptoms¹³. The source of PM₁₀ can vary, depending on the indoor furniture and occupant's activities. While environmental tobacco smoke can significantly affect the PM₁₀, present study found most assessed areas are non-smoking area. Based on visual inspection and checking to the ventilation system, most of the particulates could highly originated from dirty carpets, broken filters and supplying duct lining (Fig. 12). As majority of the ventilation filtration employed in the buildings are graded

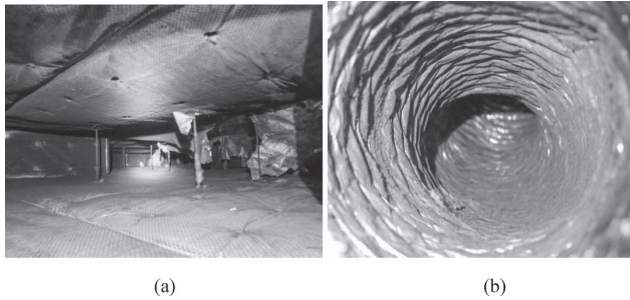


Fig. 12. Broken air duct lining (a) with accumulation of dirt and dust in the connecting supply diffuser (b).

between MERV (Minimum Efficiency Reporting Value) 5 to 8, which is generally 70% effectiveness in removing particle size between $3\ \mu\text{m}$ to $10\ \mu\text{m}$, many particulates could still be escaped and build up in the ventilation supplying and return duct.

Chemical parameters like CO were observed to be of outdoor origin. This indicated infiltration of gases and pollutants to the indoor environment as no treatment system was installed. CO₂ being the indicator for ventilation shows most sampling points were sufficiently ventilated. Formaldehyde was significantly high in places packed with furniture, old carpet and activity related to use of organic solvents. Ozone was not recorded across all sampling points. TVOC as based on American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHREA) were significantly observed in the morning but dispersed away once the MVAC was in operation. As volatile organic compounds such as benzene, toluene and xylene react to temperature and heat, they could have interact closely with other chemicals and physical parameter and change over time. Temperature, RH and air movement can significantly change the concentration of chemicals and exposure level to indoor occupants.

The bio-aerosols were found to be relatively low across all sampling points but this do not mean that occupants had low exposure to potential pathogenic biological contaminants. The current practice in the IAQ assessment only measures the total counts of microbes but do not differentiate the types of microbes, which among them could be virulent to human in small quantity. As shown in the dust sampling results, *Acanthamoeba* cysts were detected at most assessment sites. Furthermore, it is relatively low correlation between *Acanthamoeba* presences with all other studied IAQ parameters in the standard practice. This indicates that there is some other parameters to be included in the current assessment to better represent the state of being of air quality. A good IAQ assessment report should

incorporate other potential threats by opportunistic pathogen like *Acanthamoeba*. This is an area that has been overlooked under the existing IAQ ICOP.

Occupant health complaints

Most sampling points have recorded significant SBS across the studied premises. This might be due to the current IAQ practice in the country. Although the ICOP IAQ 2010 is stipulated in the Occupational Safety and Health Act, the IAQ assessment is not compulsory across the country. IAQ assessment will only be carried out when the building management received significant complaints from the occupants. This in fact provided the opportunity for the present work to be carried out. No BRI was reported to the IAQ assessor when IAQ assessments were carried out.

Ventilation system

The type of MVAC seems to affect IAQ characteristics observed at sampling sites. Centralized MVAC is observed to regulate at lower air temperature compared to individual FCU. Dust particulates in the FCU serving areas were higher due to the possible lack of ventilation capacity designed in the individual unit, and this is indicated in the higher CO₂ concentration recorded.

The present study was different from most previous ventilation system studies as it focused mainly on MVAC instead of HVAC. This is because there is no heating element in the ventilation system across all assessment sites due to tropical climate. Thus, the interaction of pollutants in MVAC system might be different from what could be found in the HVAC system. However, further studies are needed to confirm this.

Acanthamoeba as indicator for indoor air quality

Acanthamoeba cysts were found more often in the ventilation system compared to furniture dust. This could be due to the regular housekeeping regime in the office setting. Ventilation cleaning on the other hand is not common; especially those that involve ducting in the centralized MVAC.

The statistical analyses show bio-aerosol, bacteria and fungi are positively correlated to each other. Although the correlation coefficient is not strong (0.529), it might indicate some possibility of their interaction in the indoor air or MVAC. The presence of *Acanthamoeba* in the MVAC is significantly correlated to fungus count and respirable particulates in the breathing zone. This could indicate the possibility of contaminants coming from the same source or origin, such as MVAC.

The presence of *Acanthamoeba* cyst whether from MVAC or indoor environment dust, showed positive significant correlation with SBS reported by occupants of the premises. Using actual data collected as in the present study, a human experience based model is developed. The present model indicates that whenever *Acanthamoeba* cyst is detected in the ventilation system, it is five times more likely that SBS will be observed among indoor occupants. Likewise, the presence of cyst in the furniture dust correlates with 12 times more likelihood of SBS occurring. However, this does not mean a cause and effect relationship. As SBS is only defined by symptoms experienced by more than 20% of the exposed occupants, not everyone will experience SBS when cysts are detected in the MVAC. Further work is needed to determine whether the presence and types of endosymbionts in the *Acanthamoeba* cysts¹⁴⁾ in the indoor air could be an important association with SBS.

This work could be applicable as future reference for use of other protozoa study in relation to IAQ assessment. *Acanthamoeba* is used as a reference subject in the present study as it is known to be ubiquitous. It should be noted that many other types of protozoa are present in the indoor environment and ventilation system. Their role in the IAQ is relatively unknown.

Conclusion

The present study showed associations between the presence of *Acanthamoeba* cyst in the MVAC with IAQ parameters in the indoor air environment, namely total fungi count and respirable particulates, PM₁₀. The presence of *Acanthamoeba* in the MVAC has positive relationship with symptoms of SBS recorded among the exposed indoor occupants.

The detection of *Acanthamoeba* cyst could indicate a faulty MVAC in the past and the presence of possible pollutants like fungus and PM₁₀ in the environment. As the presence of cysts is significantly correlated with the symptoms of SBS experienced by occupants, it could also indicate the air quality of the indoor environment.

A logistic regression model was developed based on field data and human experiences. The model provides useful information for IAQ assessors, researchers and engineers in assessing IAQ. The present model also provides useful insight for the future use of other protozoa or any other closely related microorganisms in the MVAC system for IAQ assessment.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

SSO contributed in the conception and design of the study, carried out fieldwork and laboratory analysis and drafted the manuscript.

JWM contributed in the conception and design of the study, secured funding for the project and preparation of the manuscript.

DCKF participated in the preparing of the manuscript and revised it critically for important intellectual content.

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