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Original article

Indoor air bacterial and fungi bioburden in an electronic factory, an office and a winery in Malaysia

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Background: Microbial loads differ for different work environments and it is greatly influenced by the manufacturing processes involved.

Objectives: This study aimed to assess microbial loads and other indoor air quality parameters in selected work environments in Malaysia.

Methods: This is a cross-sectional survey carried out in an electronic factory, a winery and an office. Six sampling events were performed at all the study sites. Trypticase soy agar (TSA) (with ambient air incubation) and TSA supplemented with haemin and NADH (with CO_2 enhanced incubation) were used to isolate the non-fastidious and fastidious groups of bacteria respectively. The plates were incubated at 37°C for 3 days. Sabouraud's dextrose agar (SDA) and dichloran glycerol agar (DG-18) were used to isolate the non-xerophilic and xerophilic groups of fungi respectively. The colonies were counted and the concentrations of airborne micro-organisms were calculated as CFU/m^3 (colony forming units per cubic meter).

Results: Indoor microbial loads were generally greater indoors than outdoors at the three study sites. The electronic factory had the highest indoor microbial counts (in the order of 10^2 to 10^3 CFU/m^3 of air). All readings at the office were below the recommended level of 500 CFU/m^3 of air for offices by the Institute of Environmental Epidemiology, Singapore. The readings at the winery were also below 500 CFU/m^3 of air except for the first sampling event which coincided with the peak of production winery. Furthermore, there were significant negative correlations ($P < 0.05$) between outdoor light intensity and microbial loads. Thus, substantiates the bactericidal effect of ultraviolet light.

Conclusion: Indoor manufacturing processes are major contributors to microbial load in work environments. The presence of pathogenic micro-organisms might be potential hazard indoors; high microbial loads could therefore indicate a need for further screening. Hence, having standards for indoor microbial loads at different work environment is worthwhile.

Keywords: Indoor air, bacterial, fungi, electronic factory, office, winery.

Workplace indoor air is a major contributor to work related hazards, because indoor air quality is a significant factor for occupant's health. [1] Industrial workers may be exposed to certain airborne micro-organisms or allergens associated with a particular industrial substrate. This exposure might impact workers health as observed in a furniture factory where relatively high incidence of microbial allergic reactions was reported among furniture workers.[2] Significant sources of biological contaminants in indoor could originate not only from individuals with infections but also from indoor characteristics and entry of contaminants from outdoor sources.[3] Most microbial agents get in contact with the body through the mucus membrane and the skin (in the case of contact dermatitis.[4] When airborne spores of respirable size are inhaled, they penetrate the bronchi and alveoli, where they are lysed. Deleterious primary and secondary metabolites are produced in the process. Mycotoxins produced by some fungi could also be pathogenic when inhaled. Inhalation of airborne microorganisms could result in severe health complications.[5] Infection case by inhalation of air

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contaminants does not only depend on the type of contaminants inhaled but also on the size, the quantity inhaled, the site of deposition and current health status of building occupants.

The purpose of this study was to determine the microbial loads in the selected workplace and their relationship with other indoor air quality parameters.

Materials and methods

Description of study sites

The study sites used include an electronic factory, a winery and an office. The electronic factory and the winery are located in Kajang, Selangor while the office is located in Bukit Jalil, Kuala Lumpur. The electronic factory has a staff strength of above 100 (the precise number was not given). Both the office and the winery (factory workers) have a staff strength of 17 and 14 respectively. The electronic factory manufactures electronic products such as hard disk parts and other computer components. The operation hours were between 9 a.m. to 5.30 p.m. Various chemicals and solvents are used in the production process and of particular interest is the oil based coolant process. During the cooling process, oil aerosols are formed in the air (oil mist) and it is speculated that this could be a source of nutrient for airborne micro-organisms. Only office-based activities take place in the office selected as a study site. The major activities in the winery included the fermentation and bottling of wine. The working hours were between 8.30 a.m. to 5.30 p.m. at the office and winery.

At the electronic factory, sampling point one was at the "packaging area", this is where all finished products are packaged. The sampling point two was at the "loading area". Packaged good are transferred from the packaging area to the loading area where they are up loaded into a carrier and transferred out of the factory. Sampling points three, four, five and six were located at the "manufacturing area" where the manufacturing equipment are positioned. Sampling points seven and eight were located outdoors. Two sampling points were located inside the office while sampling point three was located outdoors. Sampling points one to four were positioned at the fermentation and filtration area at the winery. Sampling point five was at the distillation area while sampling point six was outside the winery. The number of sampling points placed at each site was determined by the floor area, based on the Department of Occupational Safety and Health's guidelines.[6]

Study design

Six sampling events were performed within nine months. The colony forming unit per cubic meter (CFU/m^3) of air, physical parameters (temperature, light intensity and relative humidity) and chemical parameters (formaldehyde and carbon dioxide) were measured for the six sampling events.

Collection of data

Data collection on the building profile of the three study-sites was undertaken by inspection and interview of the maintenance staffs and occupants of the buildings. Air samples were taken to give the best representation of the indoor air quality in the three study sites. The position of the workers and the equipment in the factories and office were considered in choosing the sampling point and position. Air samples were taken at least two hours after the commencement of work at each site. [7] Similar to the study of Chaloulakou[8], microbial samples were taken in duplicates at each point for the six sampling events. The outdoor measurements of microbial and physical parameters were targeted to access the indoor and outdoor air relationship and to provide information on the building penetration factor.

A Biomerieux™ Air IDEAL Sampler was used to collect air samples. Air particles are filtered out through the grid surface for adequate enumeration of colony forming units detected in per cubic meter (CFU/m^3). Air sample volumes ranging from 10 to 250 litres were drawn using the IDEAL Air Sampler (Biomerieux BBL Samples were collected at a height of 1.5 meters from ground level which is the normal breathing level. [7, 9 - 11] Enumeration of bacterial loads was investigated on two media. Trypticase soy agar (TSA) was used for the isolation and sub-culturing of non-fastidious bacteria. [9] TSA enhanced with hemin, NADH and CO_2 was used for the isolation and sub-culturing of fastidious bacteria. Cycloheximide was added to the medium at a concentration of 12ml/L to inhibit fungal growth. [9] After air samples were collected, the plates were transferred to the laboratory for incubation at 37°C for 3 days, while the TSA plates enhanced with hemin (5ml/L) and NADH (5ml/L) incubated in a candle jar (for CO_2 enhancement). The latter hemin, NADH and CO_2 supplemented plates were denoted TSA-Plus plates. After incubation, emergent colonies were counted and the concentrations of airborne microorganisms were calculated as colony forming units per cubic meter.

Sabouraud's dextrose agar (SDA) was used for isolation of non-xerophilic while dichloran glycerol agar **(**DG-18) was used for isolation of xerophilic fungi.[12] Chloramphenicol was added at a concentration of 0.05g/L to inhibit the growth of bacteria. After air sampling, SDA plates and DG-18 plates were incubated at 25°C and 21°C respectively for 4 days. The colonies were counted and the concentrations of airborne micro-organisms were calculated as CFU/m^3 after incubation

Light intensity was measured with a LUX/FC light meter (Tenmars, Taiwan). Temperature and relative humidity were measured with a Hygrometer (Comark, UK). An independent company was contracted for sampling of the chemical parameters. Only carbon dioxide and formaldehyde were sampled at selected points due to budget limitation. Carbon dioxide was accessed using the Kitagawa detection tube system (126B) with the measuring range of 100 ~ 1,500 ppm while formaldehyde was access with Kitagawa detection tube system (171sc) with the measuring range of 0.1 ~ 4.0 ppm

Statistical analysis

CFU/m^3 was calculated according to the IDEAL Air Sampler (Biomerieux BBL) manufacturer's manual provided. A portion of each isolate was observed under the microscope to determine if it was a bacterial or fungal isolate (for bacterial and fungal CFU/m^3 calculations respectively). The number of true isolates on each plate were then counted and converted into colony forming units per meter cube based on the volume of air samples collected, as shown below.

$$\frac{\text{Number of isolates on the plate}}{\text{Volume of air sampled}} \times 1000 = \text{CFU/m}^3$$

The number of isolates used in the calculations were the corrected values using the positive hole correction provided in the manual. The positive hole correction is required because of the possibility of having more than one isolate on a point due to the way the sampler is manufactured.

All data collected from the six sampling events at the three study sites were analysed using Excel and SPSS (SPSS. Inc., Standard Version). Pearson correlation was used to determine the relationship between microbial loads, physical parameters and chemical parameters. Correlations were significant at $P < 0.01$ and 0.05 (2 tailed).

Results

The microbial loads for all groups of microorganisms (non-fastidious bacteria, fastidious bacteria, non-xerophilic fungi and xerophilic fungi) inside the electronic factory for the six sampling events were in range of 170 to 7,300 CFU/m^3 of air and up to 625 CFU/m^3 of air outside the electronic factory. At the office, the microbial loads for all groups of micro-organisms were between 15 to 312 CFU/m^3 of air, while the readings outdoors were between 13 to 269 CFU/m^3 of air. Most readings at the winery were below 500 CFU/m^3 of air indoors and outdoors, except for the first sampling event which was the peak of production at the winery. The readings for the first sampling event in the winery were in the range of 345 to 854 CFU/m^3 of air indoors. The microbial loads outdoors for all sampling events range from 20 to 814 CFU/m^3 of air. The average CFU/m^3 of air for all the samples collected at the indoor and outdoor sampling points of the electronic factory, office and winery is shown in Figure 1.

The microbial loads inside the electronic factory were significantly higher than the microbial loads observed at other study sites indoors (Office: $P < 0.05$ for non-fastidious bacteria, $P < 0.01$ for non-xerophilic fungi and xerophilic fungi. Winery: $P < 0.05$ for non-fastidious bacteria, $P < 0.01$ for non-xerophilic fungi and xerophilic fungi). However, the microbial loads outside the electronic factory were significantly lower than the microbial loads outside other study sites for some groups of micro-organism (Office: $P < 0.05$ for fastidious bacteria, $P < 0.01$ for non-fastidious bacteria, Winery: $P < 0.05$ for non-xerophilic fungi). The office had the lowest microbial loads, significantly lower than the microbial loads observed in the winery especially for the fungi groups ($P < 0.01$ for non-xerophilic fungi and xerophilic fungi). However, the microbial load for the fastidious bacteria group outside the office was higher than that of the winery ($P < 0.01$). The microbial loads inside the electronic factory were significantly higher than the microbial loads outside ($P < 0.05$ for fastidious bacteria, $P < 0.01$ for non-fastidious bacteria, non-xerophilic fungi and xerophilic fungi). This is a bit different from the observations at other site, although the microbial load for the fastidious bacteria group was significantly higher inside the office than outside ($P < 0.01$). Xerophilic fungi load was significantly higher outdoors than indoors of the office ($P < 0.05$). The microbial loads at the winery were higher indoors than outdoors. However, only the bacterial groups showed significant

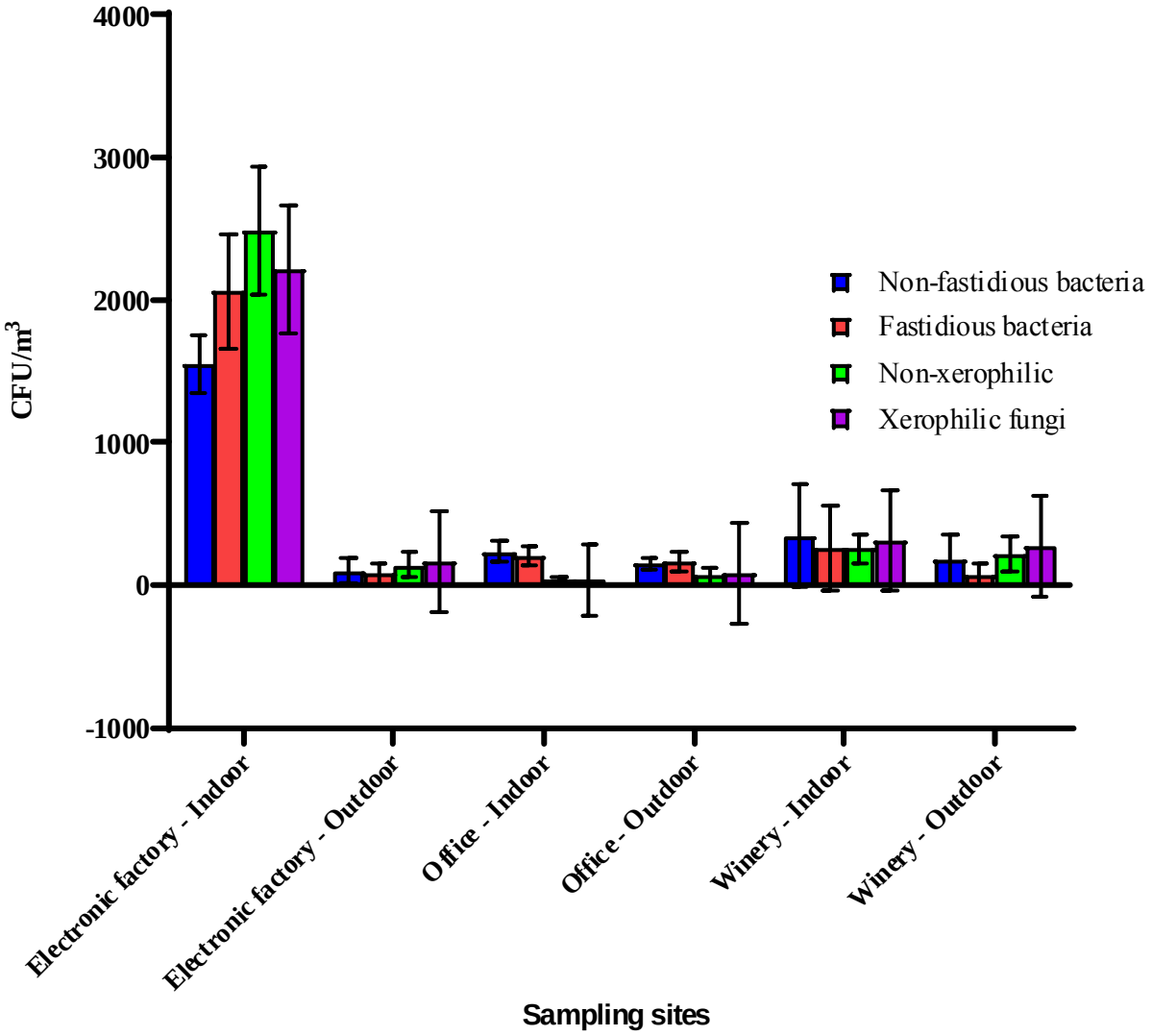

Figure 1. Average colony forming units detected in per cubic meter of air for all the samples collected at the indoor and outdoor sampling points of the electronic factory, office and winery. CFU/m^3: Colony forming units per cubic meter of air
I: Standard deviation bars

difference in the indoor and outdoor loads ($P < 0.01$ for non-fastidious bacteria, $P < 0.05$ for fastidious bacteria). The correlation analysis between the various parameters assessed at the study sites are in Figure 2. Formaldehyde was not detected at the electronic factory and office while the highest CO_2 concentration observed at the office was 800 ppm and up to 1,500 ppm in the winey. The results are shown in Table 1. The average light intensity, temperature and relative humidity at the indoor and outdoor sampling points of the electronic factory, office and winery are shown in Figure 3.

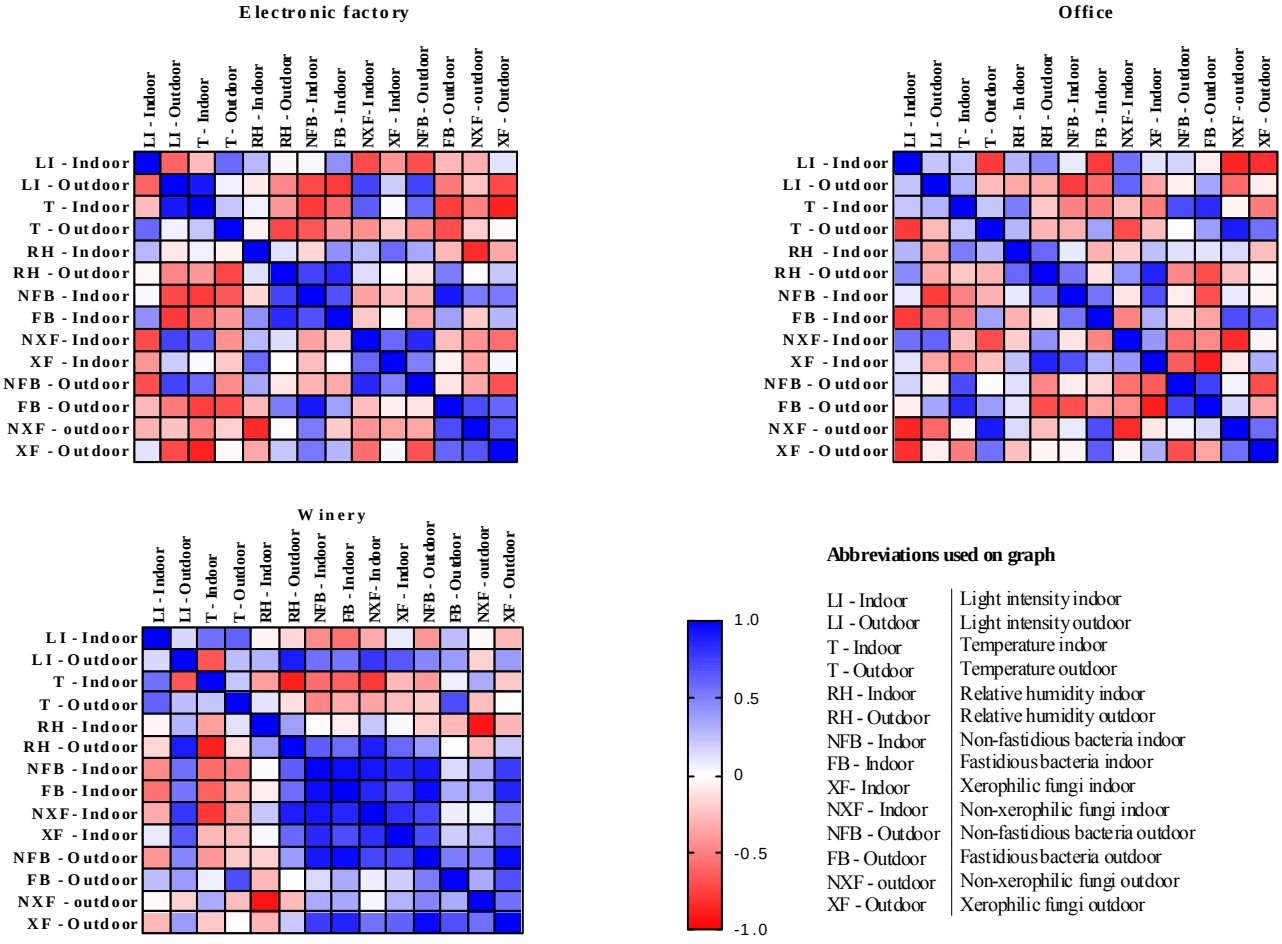

Figure 2. Correlation between the parameters assessed at the electronic factory, office and winery.

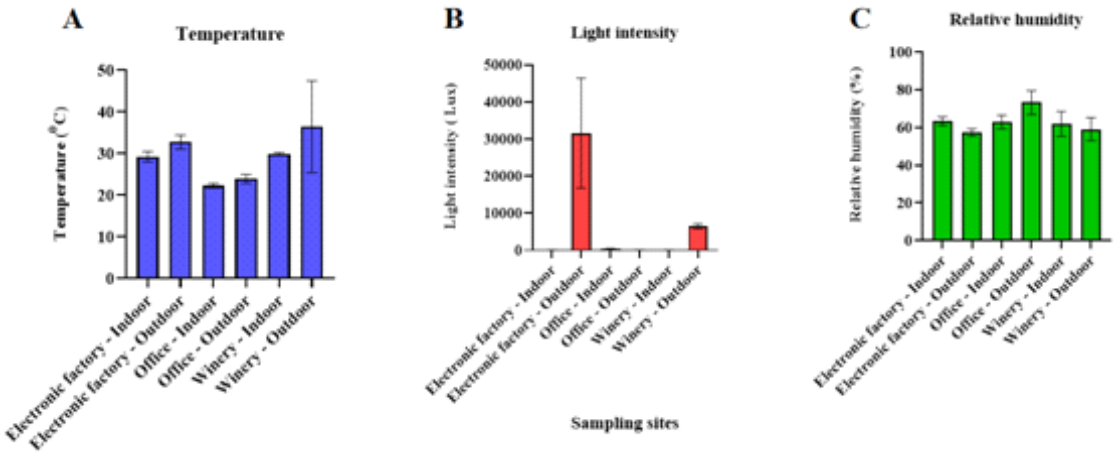

Figure 3. Average temperature (A) light intensity (B) and relative humidity (C) for all measurements taken at the indoor and outdoor sampling points of the electronic factory, office and winery. Symbol used on chart I: Standard deviation bars

Table 1. Formaldehyde and carbon dioxide concentrations (in ppm) for the six sampling events at selected points in the electronic factory, office and winery.

	First sampling		Second sampling		Third sampling		Fourth sampling		Fifty sampling		Six sampling	
	Form-aldehyde	Carbon dioxide	Form-aldehyde	Carbon dioxide	Form-aldehyde	Carbon dioxide	Form-aldehyde	Carbon dioxide	Form-aldehyde	Carbon dioxide	Form-aldehyde	Carbon dioxide
	Electronic factory											
SP 2	ND	600	ND	650	ND	500	ND	700	ND	600	ND	600
SP 4	ND	550	ND	600	ND	800	ND	800	ND	600	ND	500
SP 5	ND	400	ND	600	ND	600	ND	500	ND	600	ND	600
Office												
SP 1	ND	600	ND	600	ND	800	ND	700	ND	800	ND	800
Winery												
SP 1	ND	1,200	ND	200	ND	1,500	1	800	1	700	ND	200
SP 3	1	200	ND	700	ND	500	ND	1,500	ND	1,500	ND	600
SP 5	ND	700	ND	400	ND	400	ND	600	ND	**700**	**ND**	**500**

ND : Not detected; ppm : parts per million; SP : Sampling point

Discussion

The CFU counts at the electronic factory were distinctly higher indoors compared with the outdoor counts. Furthermore, the air volume threshold needed to isolate microorganisms outside the electronic factory was quite higher than the threshold required indoors. The higher threshold of air required outdoor was due to the comparatively lower CFU loads outdoors. This is, to large extent influenced by the bactericidal effect of ultraviolet light [13], coupled with the large air dilution effect outdoors, however only the xerophilic fungi group showed a significant negative correction with Light Intensity outdoors in this study. Many sources cite outdoor air as a major contributor to indoor contamination[4] this is not directly accountable for the high microbial loads observed in the electronic factory. The high microbial load indoors was as the resultant effect of the indoor environmental factors such as temperature and relative humidity among others. In addition, the oil based lubricant used at the electronic factory generates oil mist which possibly provides enough nutrient aerosols for some airborne micro-organisms to survive. There are few studies till date assessing the microbial quality in an electronic factory as compared with order work environments, however this study shows that the microbial loads in the electronic factory is much higher than other studied site (Figure 1). More studies are required on the microbial loads in the electronic factor and standard should be set to limit work hazarded relating to indoor air quality.

The microbial loads observed in the office are similar to other bacteriological studies.[9, 14, 15] A typical bacterial level in offices is in the order of 10^2 CFU/m^3 of air. [9] All readings for all groups of micro-organisms (non-fastidious bacteria, fastidious bacteria, non-xerophilic fungi and xerophilic fungi) were below the recommended threshold of 500 CFU/m^3 of air inside the offices: as recommended by the Institute of Environmental Epidemiology, Ministry of the Environment, Singapore. [16] Another source recommended up to 10^3 microorganisms/m^3 as the maximum safety limit, above which the air should be considered hazardous to human health. [17]

Both indoor and outdoor readings at the winery were in the order of 10^2 CFU/m^3 of air for all media of isolation. In general, the indoor air fungal loads were, greater than that of outdoor samples. Fungi tend to accumulate indoors which is in line with the study Piccco AM. and Rodolfi M. [18] The highest microbial loads for all groups of microorganisms (non-fastidious bacteria, fastidious bacteria, non-xerophilic fungi and xerophilic fungi) were observed at the fermentation and filtration area. High microbial load in food industry is a concern because of the possibility of food contamination. Although microbial contaminated from surfaces has been recognized as the main source for food contamination, airborne contamination in food industries has currently been studied. [19] Microorganisms in the floor and surface dusts indoors are said to include deposition from outdoor air [20], hence contaminated areoles can dispersed indoors and settle on exposed wine as well. Proper procedures should be adhered to in wineries to reduce wine exposure to air contaminate.

The indoor concentrations of micro-organisms might not to be stable. It could be altered by human activities at the time of sampling. Airborne fungal loads are more difficult to assess, because a multitude of new propagules are released into the environment when fungal sporangia burst, increasing the fungal concentration. [9] Moreover, fungal count fluctuations depend on the type of monitored environment. This is why multiple readings were taken over a long period of time to give a better representation of counts at each site. A positive correlation between temperature indoor and outdoor at the electronic factory indicates the direct effect of outdoor temperature on temperature indoors (Figure 2). Heightened light intensity outdoors was also associated with heightened temperatures indoors at the office (Figure 2). These are obviously due to the direct effects of ultraviolet ray. Increase in temperatures was associated with lower microbial loads inside the office (Figure 2). The temperature in the office was constantly lower compare with other study site. There was a significant positive correlation between light intensity and temperature at the winery indoors (Figure 2). Increase in light intensity was also associated with low bacterial load for the fastidious bacteria group indoor (Figure 2). Furthermore, there was a significant negative correlation between temperature and the non-xerophilic fungi indoors at this site. There were positive correlations for the fastidious bacteria group indoors and outdoors and for the xerophilic fungi group indoors and outdoors (Figure 2), these suggest the possibility of efflux of micro-organisms from indoors to immediate outdoors environment or vice vasa.

Due to budget constraints, only two chemicals i.e. carbon dioxide and formaldehyde were assessed at the three study sites. The permissible limits (by the Institute of Environmental Epidemiology, Singapore) for carbon dioxide concentration in the office environment is a maximum of 1,000 ppm but up to 5,000 ppm is allowed in industrial buildings.[16] However, Norbäck suggested that indoor concentrations of CO_2 should be below 800 ppm for optimum health.[21] The highest reading at the office was 800 ppm. Although this is acceptable, necessary preventive measures are required to maintain CO_2 concentration within acceptable limits. To maintain a good CO_2 concentration level, the air exchange rate might be increased while human density in the building might be reduced. The highest CO_2 concentration reading at the electronic factory was 800 ppm as well, which is acceptable in industrial environments while a CO_2 concentration up to 1,500 ppm was observed at the fermentation area in the winery. This was expected since CO_2 is a by-product of wine fermentation. A study by Norbäck D, *et al*. shows a significant positive correction between indoor CO_2 concentrations and ocular symptoms, nasal symptoms, throat symptoms, shortness of breath, headache, tiredness and dermal symptoms.[21] Formaldehyde was not detected at the electronic factory and office. This does not necessary suggest the absence of formaldehyde, it may present at a concentrations below the detectable limit. The recommended formaldehyde concentration indoors is 0.1 ppm. Formaldehyde concentration was 1.0 ppm at sampling points one and three at the winery (fermentation area), i.e. above the recommended levels. Formaldehyde has been associated with eye irritation at thresholds of 0.01 - 2.0 ppm and upper respiratory symptoms at thresholds of 0.1 - 2.5 ppm.[7]

Conclusion

Indoor manufacturing processes are major contributors to microbial load in work environments. The presence of pathogenic micro-organisms might be potential hazard indoors. Hence, indoor air screening should not be limited to microbial loads, micro-organism identification and profiling should be included. Investigators might proceed with microbial identification if the microbial level exceeds the recommended. Indoor air screening should also be incorporated into indoor air quality guidelines. Furthermore, meteorological changes should suggest a need for indoor air screening, since there are significant corrections between microbial counts and relative humidity, temperature and light intensity. Industrial building and workplace should maintain healthy ventilating system as accumulated dust on air conditioning filters and surfaces may be sources of airborne bacteria and fungi.[22] Hence long-term regular assessments of indoor air or settled dust are also recommended.[23] Overcrowding should be avoided in workplace to maintain healthy breathable air indoors.

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Conflict of interest

The authors, hereby, declare no conflict of interest.

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