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Particulate Matter ($PM_{2.5}$) and Mould Characteristics in Selected Classrooms Located in Waikato, New Zealand: Preliminary Results

Kaia Williams [1], Rhys J. Jones [1] and Mohammad Al-Rawi [2,3,*]

[1] Centre for Applied Science and Primary Industries, Te Pūkenga-Waikato Institute of Technology, Hamilton 3240, New Zealand; kaiwil34@student.wintec.ac.nz (K.W.); rhys.jones@wintec.ac.nz (R.J.J.)
[2] Centre for Engineering and Industrial Design, Te Pūkenga-Waikato Institute of Technology, Hamilton 3240, New Zealand
[3] Faculty of Engineering, Chemical and Materials Engineering, The University of Auckland, Auckland 1010, New Zealand
* Correspondence: mohammad.al-rawi@auckland.ac.nz

Abstract: Small airborne particulate contaminants such as mould spores can harm human health by causing or exacerbating respiratory illnesses. Such particulates tend to be microscopic; however, in the case of moulds, contamination can be associated with visible colonial growth on surfaces and musty odours detectable by occupants of the room. Shared spaces, such as offices and classrooms, represent areas of higher risk due to the larger numbers of people being exposed to airborne particulates. To better appreciate the health risks associated with airborne particulates, it is therefore advantageous to assess the levels of breathable particulates in a room and compare them with the proportion of particulates represented by mould spores. An air image sensor machine was used to collect $PM_{2.5}$ particulate levels for three urban-campus classrooms and three semi-urban-campus classrooms during different wintertime (August) days in New Zealand. For each room, a settle-plate method was also used to compare background mould levels at breathing height for seated occupants. Three of the classrooms had been recently built or renovated with an adequate ventilation system installed, while the remaining three classrooms were not upgraded and had no evidence of a ventilation system. The results indicated that the classrooms in the new building, located at the semi-urban campus, tended to have lower levels of particulate matter $PM_{2.5}$ compared with the urban classrooms, which had not been upgraded. However, the semi-urban classrooms tended to have higher mould counts than the urban spaces. Moreover, the building envelope for both new and old classrooms tended to be porous, with indoor $PM_{2.5}$ readings increasing in step with outdoor $PM_{2.5}$ readings. This study will assist in identifying new approaches to reduce the risk of particulate-related respiratory issues associated with urban teaching spaces, particularly those buildings requiring more sustainable technologies to purify the air and improve the indoor air quality (IAQ).

Keywords: classroom atmospheric characteristics; indoor air quality; fungi; particulate matter ($PM_{2.5}$)

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1. Introduction

Since the start of the COVID-19 pandemic, governments and the private sector have introduced a range of new policies that aim to reduce the risk of commercial building users acting as sources of microbial infection for other users of those spaces [1–4]. The education sector represents a particularly high-risk group of building users, due to large numbers of learners commonly spending extended periods of time together in open lecture rooms. An example of how this risk has been addressed in the education sector can be seen in China, where many universities moved to online and hybrid teaching during the pandemic period [5,6]. However, complete separation of learners from a collective learning space is not always possible or practicable, so measures to improve the hygiene

of learning spaces have also needed to be introduced. In New Zealand, the Ministry of Education has introduced guidelines to the early learning sector for the cleaning of teaching spaces to reduce the risk of spreading the SARS-CoV-2 virus (the causal agent of COVID-19 disease) [7]. Guidelines for cleaning surfaces within learning spaces, however, represent only part of an effective solution because, like many pathogenic viruses, SARS-CoV-2 is an airborne pathogen capable of remaining suspended in the air on particles for many hours [8], thus representing an ongoing infection risk to occupants of that room. For example, it was observed that SARS-CoV-2 RNA can be detected on particles as small as 2.5 μm, and such particles were observed to travel up to one metre from their sources [9]. It has also been noted that the suspension behaviour of particles smaller than 5 μm in diameter is like that of pollens [8]. It therefore follows that the monitoring and removal of small airborne particles in teaching environments will reduce the exposure of room users to viruses that employ such particles as transmission vehicles, as well as reducing exposure to the particles themselves, be they pollens, mould spores, dust, smoke or other small particles associated with respiratory diseases generally.

Small particles, such as dust, dirt, soot, smoke and mould spores cannot generally be seen with the naked eye. However, the monitoring of such particles is important due to their association with negative health impacts, and, consequently, they are a focus of ongoing particulate matter (PM) research by leading institutions, such as the United States Environmental Protection Agency [10]. The PM ranges of particular interest are $PM_{2.5}$ and PM_{10}, because these sizes are inhalable. $PM_{2.5}$ is classified as fine particles with diameters of 2.5 μm or smaller, while PM_{10} includes particles with diameters of 10 μm or less [10]. As noted, $PM_{2.5}$ and PM_{10} particles are also those associated with an ability to suspend respiratory viruses in the air for long periods and carry them for considerable distances.

The term 'sick building syndrome' (SBS) describes a situation associated with poor indoor air quality (IAQ) in which building occupants have negative health effects where, while appearing to be connected to time spent in a building, no specific illness can be identified [11,12]. Widely cited reasons for SBS include inadequate ventilation and poor air quality because they promote the growth and spread of the types of moulds that can create and spread small particles related to mycotoxins, allergens, irritants and pollutants [11,13–16].

It is important to also consider the contribution of outdoor air quality on IAQ. Whilst semi-urban and rural areas may have less pollution caused by industrial emissions, in general it has been found that urban areas may have lower particulate levels than semi-urban or rural areas; however, the sources of particulate matter vary. For example, in a study of Polish kindergartens by Błaszczyk et al. (2017), air samples were taken from kindergartens located in both a rural area and an urban area over a 24 h period using a Sequential Dichotomous Partisol-Plus (Model 2025) sampler (for outdoor air samples) and a PNS-15 sampler (Atmoservice LVS, air volume 2.3 m^3/h) for indoor air samples [17]. The study concluded that there was a difference in IAQ between rural and urban areas and that the pollutants at each site had different sources, highlighting the idea that when ensuring adequate IAQ within indoor environments, different sets of factors need to be considered, such as gaseous pollutants from the atmosphere around urban kindergartens, as opposed to particulate pollutants generated by coal stoves used to prepare meals in rural kindergartens [17].

Yeasts and moulds (fungi) are heterotrophic eukaryotic microorganisms that obtain nutrients through absorption [18]. They thrive in wet, poorly ventilated places and grow best in the cooler months due to moisture and humidity [18,19]. Most fungi reproduce asexually by distributing spores (conidiospores, sporangiospores and arthrospores) through water, animals and/or people. Fungal taxonomy is complex and is constantly changing as new ways of classifying its members are developed. Generally, the types of fungi associated with respiratory illnesses are related to three groups of fungi: Mucoromycota, which includes bread moulds such as *Mucor* and *Rhizopus*; Ascomycota, which includes yeasts and sac fungi such as *Aspergillus* and *Penicillium*, as well as *Stachybotrus*, which has been associated with SBS; and Basidiomycota, which includes the respiratory pathogen *Cryptococcus*.

Such divisions are based on how the fungus reproduces [18,20]. Fungi can reproduce via fragmentation, budding, spore production or sexually through homothallic/heterothallic mycelia [18,20]. Some fungi can produce irritants, allergens and mycotoxins that can lead to sinus irritation, respiratory infections, nausea, gastrointestinal disturbances and vomiting [18,20].

Of particular importance to buildings in New Zealand is the species *Stachybotrys chartarum*, which is often found in damp houses and is believed to be associated with a range of respiratory illnesses. *Stachybotrys* is a genus of ascomycete that reproduces asexually by producing spores in sticky masses of conidia. *S. chartarum* appears as a greenish black mould, reflecting the colour of the conidia contained within the slime heads. As with other ascomycetes, the ascus disintegrates explosively, ejecting the spores after maturity. This type of ejection method is extremely effective at dispersing the spores widely over a short period of time [21]. The species favours growth in damp areas rich in cellulosic substrates, such as the internal walls of houses, and is known for its ability to generate toxic macrocyclic trichothecenes and haemolytic stachylysin, which causes mycotoxicosis and respiratory tract symptoms such as nasal irritation, burning and congestion, chest tightness and dyspnea [22,23].

Given the relationship of particulate matter with respiratory illness, including the contribution of moulds in New Zealand buildings, the aim of this study was to make a preliminary assessment of mould and particulate matter $PM_{2.5}$ profiles at a New Zealand educational institute and determine the potential health implications associated with urban and semi-urban classrooms evaluated during the damper and cooler part of the year. As part of addressing the study's aim, we compared data collected from untreated classrooms with data collected from classrooms treated with an air cleaning device (dehumidifier with HEPA filter), as suggested by the Ministry of Education of New Zealand, to identify the device's impact on particulate matter and mould in the room.

2. Materials and Methods

2.1. Particle and Mould Assessment

In this study, monitoring of viable mould particles was undertaken using a settle-plate method, described by Pasquarella et al. (2000) [24]. Briefly, Petri dishes containing Sabouraud Dextrose Agar without antibiotics (SDA; Difco, Sparks, MD, USA) were exposed to the air within each evaluated room for an amount of time determined with an initial optimisation test, then incubated aerobically for five days at 25 °C. Colonies were counted and results expressed as average colony-forming units (cfu) produced per plate per unit of time exposed.

To determine the levels of particulate matter in the air, $PM_{2.5}$ levels were measured using an air image sensor placed in the classrooms for the period of the experiments. The sensor was placed in the middle of the room at height of 1 m. The Camfil AirImage sensor used was manufactured by Camfil AB, Stockholm, Sweden. The sensor has a weight of 200 g, dimensions of (W = 144 × H = 64 × D = 61 mm). Operation requires 230 V alternating current > 5 V direct current and 10 W consumption. The sensor can measure PM2.5 and PM1, with an operating range of 0 to 100 $\mu g/m^3$ and accuracy of ± 0.1 $\mu g/m^3$. For temperature, the accuracy is ± 0.5 °C with an operating range −10 °C to +50 °C. For relative humidity percentage (%RH), the accuracy is $\pm 2.5\%$ with an operating range 0 to 100 non-condensing.

2.2. Location of Study Rooms

In this study, we investigated three classrooms at Waikato Institute of Technology (Wintec) City Campus, located in the centre of Hamilton, and three classrooms at the Wintec Rotokauri Campus, which is located at the rural fringe of Hamilton, and can hence be classed as semi-urban. Hamilton is a city of approximately 180,000 people, located in the Waikato region of New Zealand. These two campuses are shown in Figure 1, where in Figure 1a the paucity of green space due to the urban environment contrasts significantly

with Figure 1b, which has a semi-urban environment. The three classrooms selected at the City campus were identified as A1, A2 and A3, which have the following air volumes: 117.5, 117.5 and 215.2 m^3, respectively. Classrooms A1 and A2 are heated with an old HVAC system, operating with relative humidity measures of 49.5% and 51%, respectively, and room temperatures of 20 °C and 21 °C, respectively. The classrooms selected at the semi-urban campus were identified as B1, B2 and B3, which have the following air volume: 275.2, 66.2 and 62.7 m^3, respectively. Classroom B1 has no heating or mechanical ventilation system in it and has relative humidity of 55% and room temperature of 14 °C. Classrooms B2 and B3 are heated with an older HVAC system and operate with relative humidities of 46.3% and 41.7%, respectively, and room temperatures of 18.5 °C and 20 °C, respectively. All rooms had doors and windows closed while the device and sensor were operating. Three of the six classrooms (A3, B2 and B3) possessed new or upgraded ventilation systems. In contrast, the remaining classrooms (A1, A2 and B1) had older ventilation systems, and there were visible signs of mould growth inside the rooms. Classroom B1 was of particular interest as it was observed to have a musty odour upon entry, suggesting it may contain favourable conditions for mould growth.

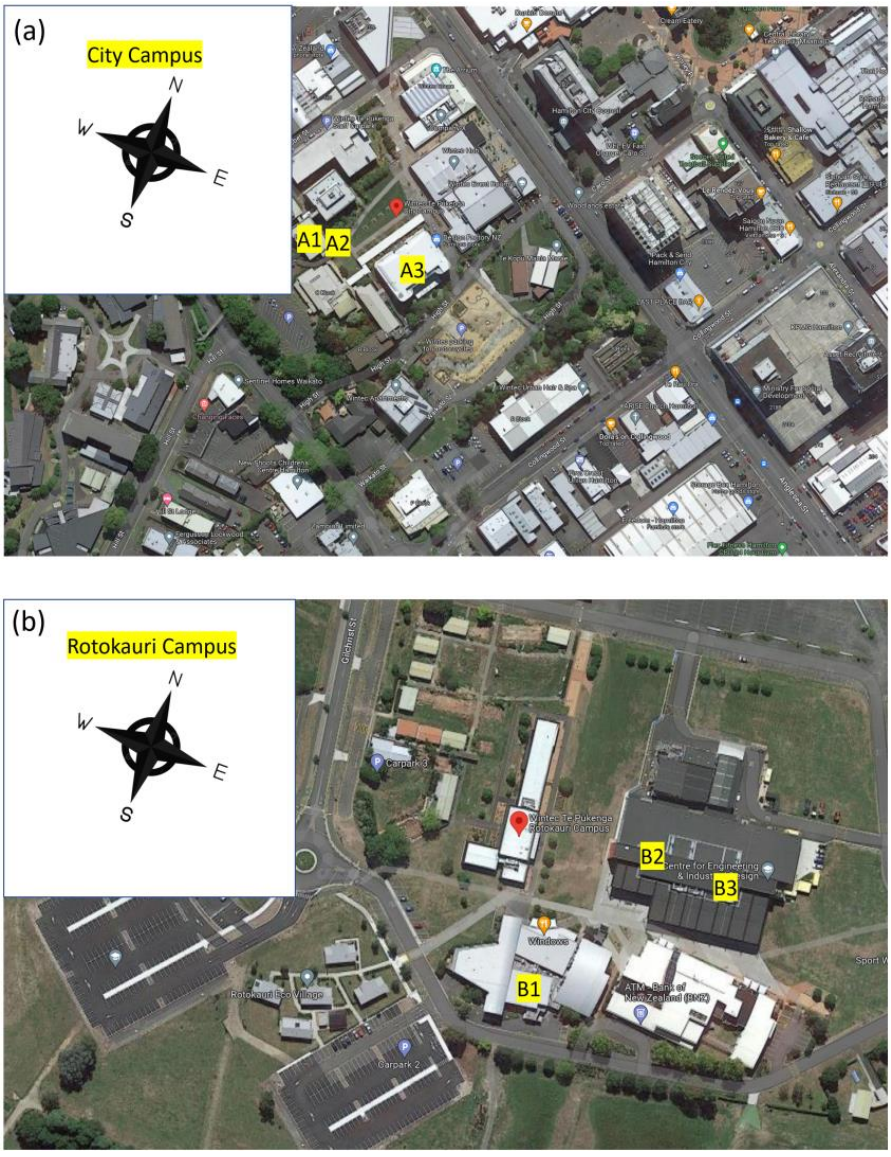

Figure 1. Study location. (**a**) The City campus, (**b**) the Rotokauri campus, including the location of each of the assessed rooms.

2.3. Settle-Plate Exposure Time

Optimum exposure times for SDA plates (settle-plates) vary depending on the properties of the spaces being assessed and can range from as little as 15 min to as much as 4 h [24]. To determine the optimum exposure time for the rooms under assessment, an initial trial was performed to compare mould counts on plates exposed for three different time periods. Three SDA plates were placed in each of three of the four corners of our laboratory (nine plates in total), which forms part of the Wintec City Campus. The lids were removed and the surfaces of the plates exposed to the atmosphere for either 1 h, 2 h or 3 h durations. During the three-hour period, a preliminary particulate matter assessment was also undertaken using the Camfil Sensor (described in Section 2.1) placed in the centre of the room. After each time period was completed, the lids were replaced on the relevant plates and the plates were incubated aerobically for five days at 25 °C. The results of this initial test indicated that a 2 h exposure was a suitable duration to allow a useful number of colonies to form on the exposed plates.

2.4. Data Collection

Each of the six study rooms was examined using duplicated SDA plates. Eight plates were used for inside each room, and these were placed spatially at each of four corner areas (two per corner), at a height of 1 m in each corner location. The rooms did not have open windows. For outdoor measurements, duplicate plates were placed beside the outside wall of the building at a point closest to the internal room being assessed (described as position "o" in the Results section). Position "o" represented the closest point outside of the building to the geometric centre of the room being assayed for fungal populations. The primary reason for measuring fungal populations at Position "o" was to obtain an appreciation of the fungal spore 'pressure' outside the building, as this may have an impact on spore levels within the building. The Camfil air image sensor was placed in the middle of the room and set to collect $PM_{2.5}$ data as described in Section 2.1 above. Plates were placed on pedestals of approximately 1 m height (to reflect the average height of seated classroom occupants and, therefore, the position from which they would be inhaling the air) above the finished floor level. The plate lids were removed to expose the agar surface to the air. The doors were then locked so that the room was not disturbed. After 2 h exposure, the lids were replaced, and the plates incubated aerobically for five days at 25 °C. Fungal colonies were then counted. In a separate study, several 'tape-tests' were performed by pressing pieces of adhesive tape on the internal room walls. The impressed tapes were pressed onto microscope slides and examined microscopically for fungal material after staining with lactophenol cotton-blue stain. Due to availability of the air image sensor, the study rooms were assessed during the period 2 August to 26 August 2022.

The above study was repeated two weeks later (between 16 August and 26 August) after rooms had been pre-treated using a modified dehumidifier (model: DB48WH-NZGC (Camfil AB, Stockholm, Sweden) with fan flow rate of 0.018 m^3/s) using two types of filters (details about the device are available in [25]). Briefly, the incoming air was filtered using a Dual-10 30/30 (ASHRAE MERV 8) polyester filter with air flow of 1700 m^3/h and, after dehumidification, returned to the room through a CityPleat-200 (ASHRAE MERV A 7A) filter with air flow 1500 m^3/h (see Figure 2 and Table 1). Pre-treatment of the rooms consisted of placing the modified dehumidifier in the classroom after 17:00 and running continuously before switching off and placing settle-plates the next day at 07:00 for two hours exposure as described earlier.

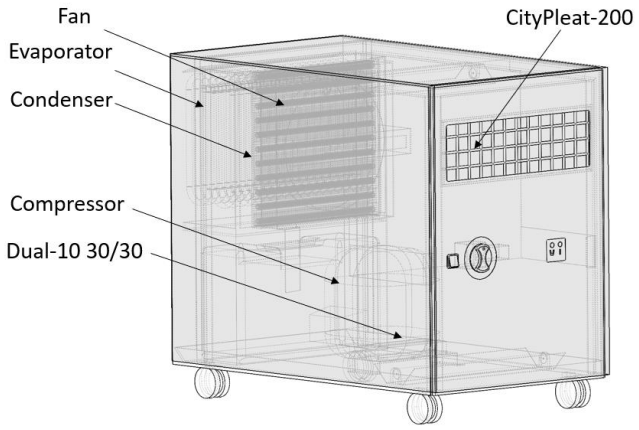

Figure 2. Schematic 3D drawing of the modified dehumidifier device.

Table 1. Filter specifications used in the modified dehumidifier device.

Filter Type	Dual-30/30	CityPleat
Filter Dimensions (Width, Height, Depth)	(289, 594, 44) mm	(289, 594, 44) mm
ASHRAE Filter Class	MERV 8	MERV A 7A
Material	Polyester	Synthetic fibre
Operation Conditions Max. Temperature Humidity	 70 °C 100%	 0–40 °C 30–70%
Weight and Initial Pressure Drop	0.6 kg, 70 Pa	0.9 kg, 135 Pa
Airflow	1700 m^3/h	1500 m^3/h

3. Results

The aim of this study was to compare mould populations and particulate matter between urban and semi-urban classroom environments. The study was performed in different classrooms located at two different campuses. We evaluated four sites in each classroom and one site immediately outside the building. Settle-plate results for each of the six classrooms are presented in Figures 3–9. Results are presented as log mould counts and particulate matter ($PM_{2.5}$).

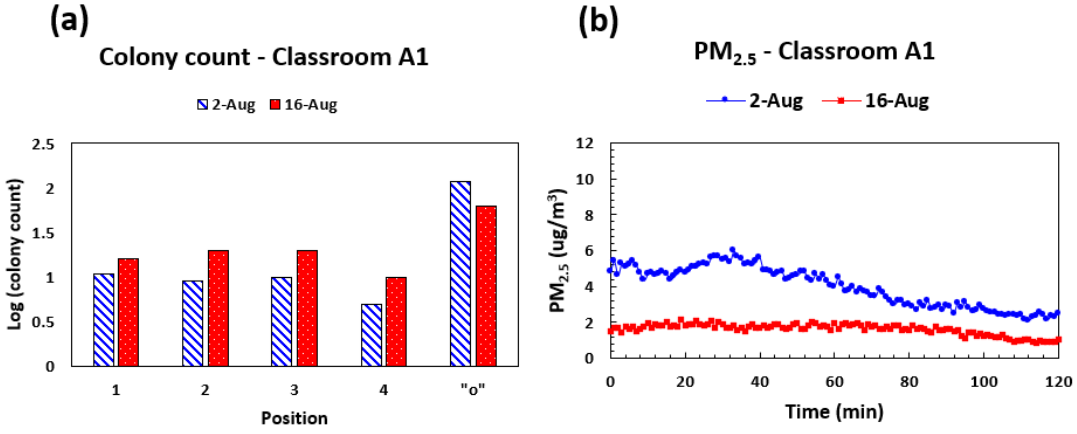

Figure 3. Classroom A1 (**a**) mould counts (log count/2 h exposure) and (**b**) particulate matter $PM_{2.5}$ ($\mu g/m^3$) during the morning period (07:00–09:00) where the 16 August data were obtained after the air cleaner had been run for 14 h.

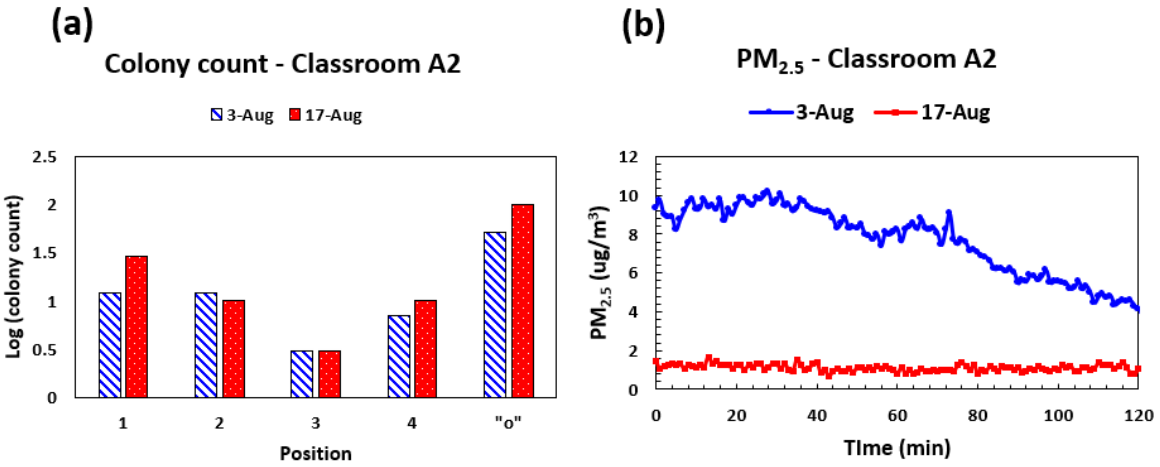

Figure 4. Classroom A2 (**a**) mould counts (log count/2 h exposure) and (**b**) particulate matter $PM_{2.5}$ ($\mu g/m^3$) during the morning period (07:00–09:00) where the 17 August data were obtained after the air cleaner had been run for 14 h.

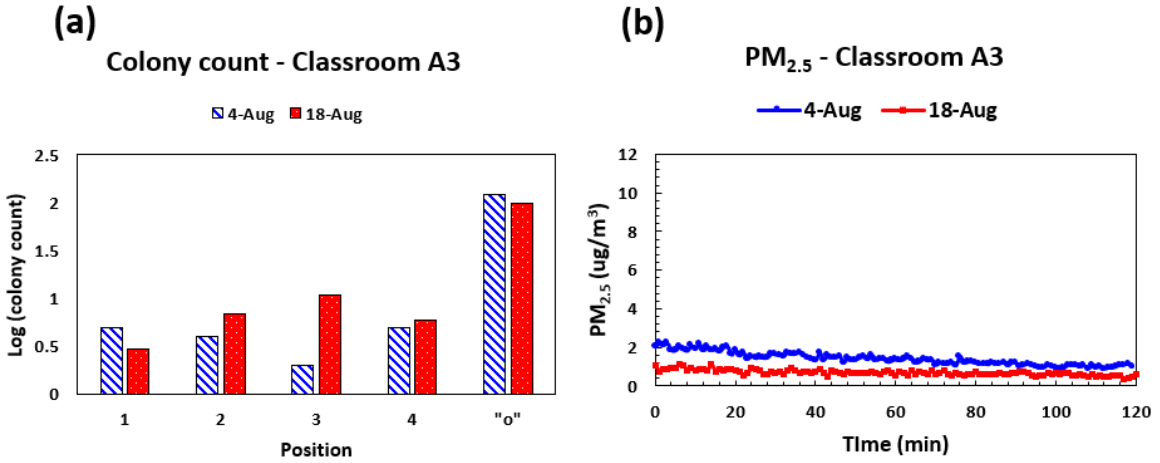

Figure 5. Classroom A3 (**a**) mould counts (log count/2 h exposure) and (**b**) particulate matter $PM_{2.5}$ ($\mu g/m^3$) during the morning period (07:00–09:00) where the 18 August data were obtained after the air cleaner had been run for 14 h.

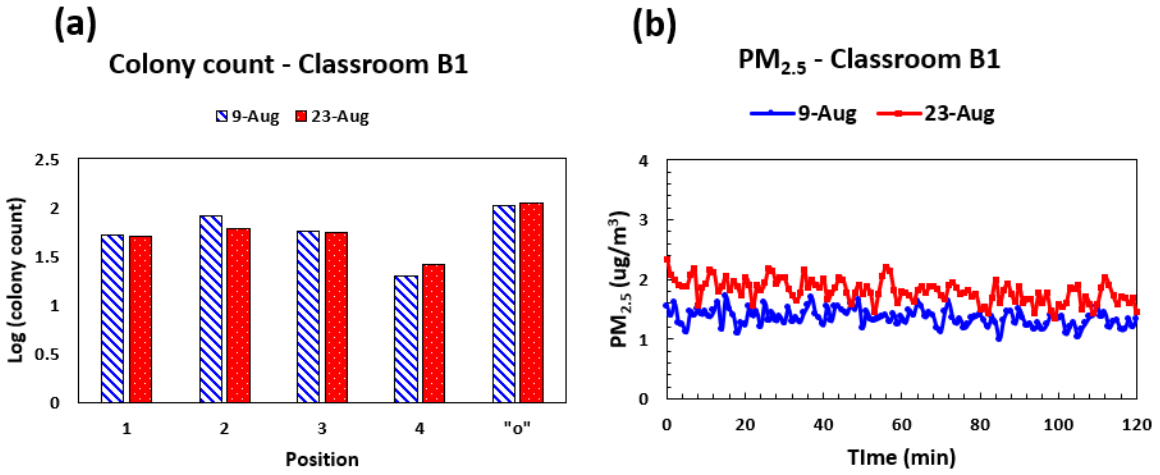

Figure 6. Classroom B1 (**a**) mould counts (log count/2 h exposure) and (**b**) particulate matter $PM_{2.5}$ ($\mu g/m^3$) during the morning period (07:00–09:00) where the 23 August data were obtained after the air cleaner had been run for 14 h.

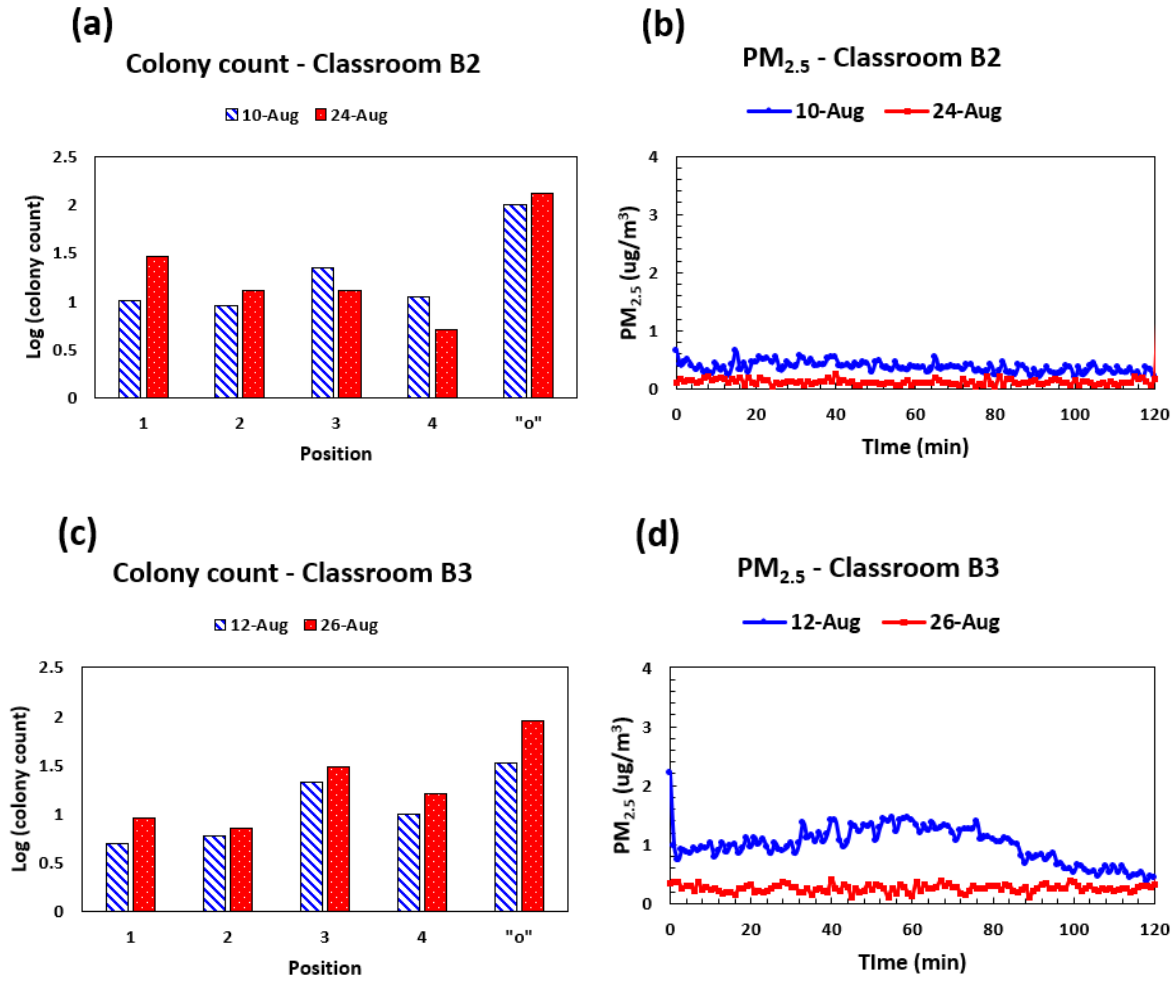

Figure 7. Classroom B2 (**a**) mould counts (log count/2 h exposure) and (**b**) particulate matter $PM_{2.5}$ ($\mu g/m^3$) during the morning period (07:00–09:00) and Classroom B3 (**c**) log mould counts and (**d**) particulate matter $PM_{2.5}$ ($\mu g/m^3$) during the morning period (07:00–09:00) where the 26 August data were obtained after the air cleaner had been run for 14 h.

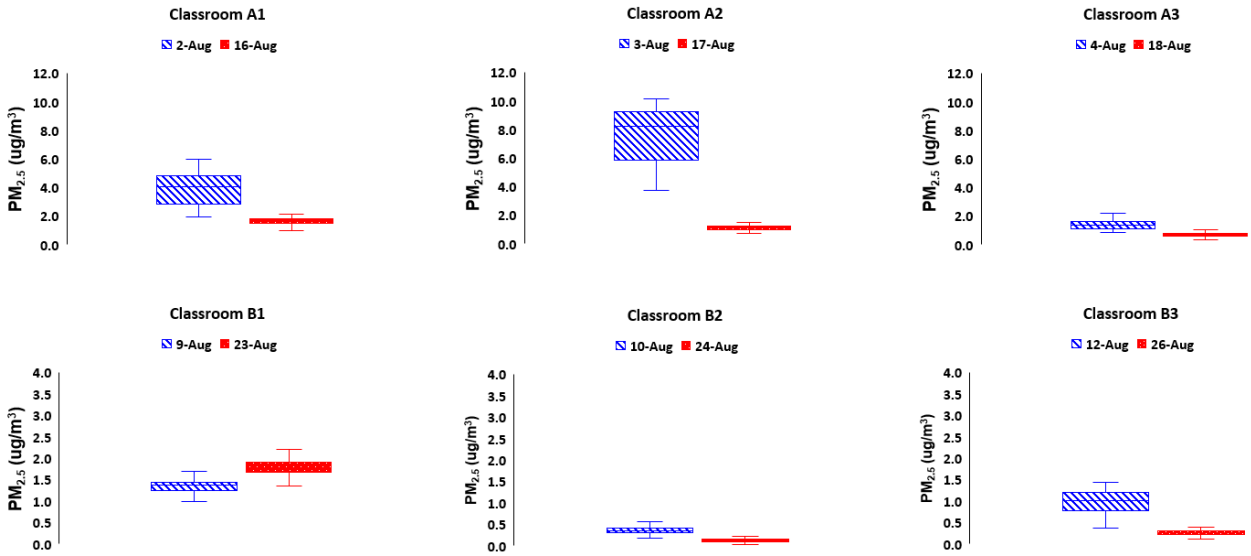

Figure 8. Box and whisker plots for $PM_{2.5}$ for all classrooms.

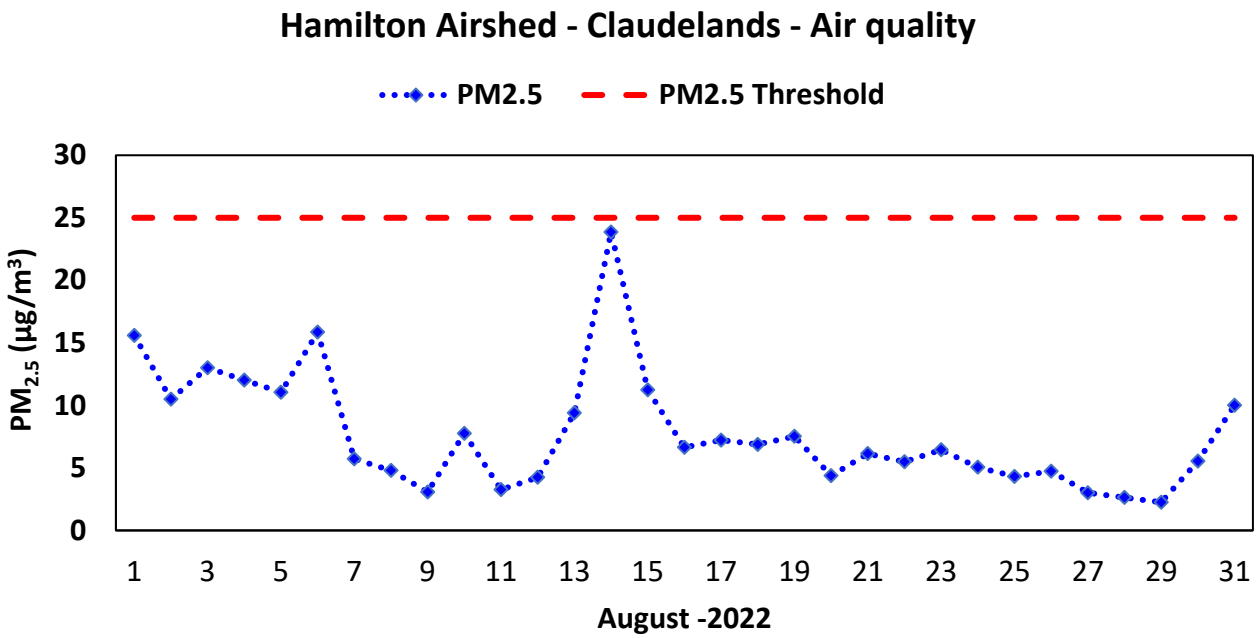

Figure 9. The particulate matter ($PM_{2.5}$) readings for Hamilton City during August 2022.

3.1. City-Based Classrooms

Figure 3 shows colony counts for classroom A1 during the two periods 2 August (no pre-treatment) and 16 August (with pre-treatment). Mould counts were higher on 16 August compared with 2 August. In contrast, 16 August average particulate matter concentrations were 59% lower than those for 2 August, as described in Table 2. This indicates the effectiveness of air cleaners to purify the air by reducing particulate matters; however, whilst air filtration will remove airborne mould spores, these findings support the view that pre-treating air will not generally have a significant inhibitory effect on the emitting source of mould growth in the classroom.

Table 2. Changes in $PM_{2.5}$ concentrations for classrooms A1–B3.

Classroom	Date	Average $PM_{2.5}$ (µg/m^3)	Change in Average $PM_{2.5}$ Concentration (%)
A1	2 August 2022	3.93	−59
	16 August 2022	1.62	
A2	3 August 2022	7.64	−85
	17 August 2022	1.11	
A3	4 August 2022	1.39	−50
	18 August 2022	0.70	
B1	9 August 2022	1.36	33
	23 August 2022	1.81	
B2	10 August 2022	0.37	−64
	24 August 2022	0.13	
B3	12 August 2022	0.98	−73
	26 August 2022	0.26	

Figures 4 and 5 show a similar trend for moulds and PM2.5 in classrooms A2 and A3, obtained on 3 and 17 and 4 and 18 August, respectively. The results again support the argument that air cleaners are effective in reducing particulate matter, as suggested by many manufactures, as shown in Table 2, with post-treatment levels showing an 85% and 50% reduction in average $PM_{2.5}$ concentrations in A2 and A3, respectively. However, there

was no improvement with respect to reducing colony counts generated by moulds in rooms. Classrooms A1–A3 (Figures 3–5) also indicate that while outdoor mould counts appear to be slightly higher than indoor counts on any given test day, the differences between indoor and outdoor counts are minimal for all but room A3.

The test results from classrooms located in the urban-based campus indicated that indoor mould and particulate matter readings are not correlated. For example, while indoor mould counts tended to range between 0.5 and 1.0 log per 2 h exposure for each of the three test rooms A1–A3, either with our without pre-treatment using the modified dehumidifier, particulate matter counts differed more widely between no treatment and pre-treated rooms, as well as between rooms where initial particulate matter levels ranged from approximately 2 $\mu g/m^3$ (room A3) to 10 μg $/m^3$ (room A2). Whilst these higher levels of particulate matter could possibly be due to unknown factors, such as the clothing of students attending the classrooms, cleaning schedules, and age of the building, it is likely a feature of higher particulate matters in an urban environment, where increased population in the urban area correlates to an increase in pollution [26]. There is potentially also a relationship between particulate matter levels and the lower mould counts: where the mould count is lower, there are fewer airborne mould spores which would be detectable by the particulate matter sensor.

3.2. Semi-Urban Classrooms

Figure 6 presents mould counts and particulate matter obtained for study room B1 at the semi-urban Rotokauri campus on 9 and 23 August. This classroom had previously had numerous complaints about having a 'bad' smell and noticeable moisture build-up on the walls. Interestingly, the mould counts in classroom B1 tended to be higher than for any of the three City-based classrooms, as well as for the remaining semi-urban rooms, and the average $PM_{2.5}$ concentration was actually 33% higher post treatment, unlike the trends seen for the other rooms, and the reason for this difference was unclear.

Figure 7 shows the mould and $PM_{2.5}$ for classrooms B2 and B3 during the following periods, 10 and 24 August and 12 and 26 August, respectively. Overall, the results indicated that mould counts for semi-urban classrooms ranged between 85 and 307 cfu/2 h exposure during the first and second week of August. The $PM_{2.5}$ concentrations, post treatment, fell by 64% and 73% for B2 and B3, respectively, as shown in Table 2. The outdoor mould counts tended to be around 200 cfu/2 h for the same period. It is noteworthy that outdoor mould counts tended to be similar between the City and Rotokauri sites; however, mould counts tended to be lower in City classrooms. Data collected in weeks 3 and 4 for the month August indicated that the mould count for week 3 was 145 cfu/2 h exposure inside and 260 cfu/2 h exposure outside for urban classrooms. However, the week 4 results show 316 cfu/2 h exposure inside and 333 cfu/2 h exposure outside for semi-urban classrooms. The Waikato Regional Council shared their particulate matter ($PM_{2.5}$) data for the month of August 2022 [27], which indicated that, during the experimental period, the $PM_{2.5}$ levels were above the threshold of 25 $\mu g/m^3$ on the following days: 2–5 August and 13 and 14 August, as shown in Figure 8. These data support the observation that the indoor $PM_{2.5}$ values were lower than the outdoor ones during the data collection in these classrooms. This explains the high readings of $PM_{2.5}$ on the first week, as shown in Figure 4b where the $PM_{2.5}$ reaches 10 $\mu g/m^3$. Additionally, the outdoor $PM_{2.5}$ for 14 August was recorded as 23.85 $\mu g/m^3$. However, the same impact was not observed on the days of the 16 and 17 August, where 14 and 7 $\mu g/m^3$ were recorded, respectively, whereas after the weekend the indoor readings were below 2.5 $\mu g/m^3$ for classrooms A1 and A2, as shown in Figure 8. Our results, as illustrated in Figure 8 and Table 2, show that the $PM_{2.5}$ concentrations dropped in all except classroom B1 at the semi-urban campus.

Figure 9 shows the $PM_{2.5}$ readings provided by the local municipal authority (Hamilton City Council) during August 2022. During that month, the Figure shows that the readings did not exceed the threshold of 25 ($\mu g/m^3$), with the highest reading occurring on 14 August, with a value of 23.85 ($\mu g/m^3$). These readings are not atypical for a smaller

city like Hamilton, New Zealand, which has relatively low pollution and gas emissions. The airshed station is located approximately 1.8 km away from the urban campus and around 9.9 km away from the semi-urban campus under investigation.

The outdoor PM2.5 readings provided by the local municipal authority (Hamilton City Council) [27] at 9 am for the days of the experiments are shown in Table 3.

Table 3. Outdoor PM2.5 concentrations at 9:00 am (Hamilton City Council).

Classroom	Date	PM2.5 (μg/m^3)
A1	2 August 2022	7.2
	16 August 2022	6.7
A2	3 August 2022	21.7
	17 August 2022	7.2
A3	4 August 2022	3.9
	18 August 2022	4.6
B1	9 August 2022	2.5
	23 August 2022	6.7
B2	10 August 2022	2.3
	24 August 2022	5.3
B3	12 August 2022	2
	26 August 2022	4.3

4. Discussion

The main purpose of this study was to collect and analyse mould populations and particulate matters of 2.5-micron diameter or less to improve our understanding of the quality of breathing air supplied to students in six classrooms over selected days during the winter period. Wintec classrooms at both the urban (City) campus and the semi-urban Rotokauri campus of Wintec were assessed, using duplicate tests across four positions within each room, to determine whether the perceived "fresher" outdoor air at the semi-urban campus made any difference to the levels of moulds and particulate matter. The settle-plate method was used for this study as it is widely accepted for good manufacturing practice (GMP) assessments of work room air quality, and our exposure times and incubation temperatures would be unlikely to result in moisture loss or other changes to media composition that could negatively impact mould recovery [28].

One classroom was chosen for its noticeable musty odour at the semi-urban campus, but no similar classroom was identifiable in the urban campus. Interestingly, in a separate study, tape-tests revealed spores to be present on the walls of classroom B1 (Figure 10). However, spores were not detected in other rooms using this method, suggesting there may be a link between the presence of high levels of spores and the musty smell observed in room B1. These findings reflect similar observations of other workers reporting a positive correlation between visible fungi and high fungal spore counts in teaching spaces [29].

The classrooms studied had hosted a variety of different classes with a variety of different people who all can be assumed to have originated from different dwellings. Because of this, different types of fungi were likely brought into the room by the people who had used the classroom prior to the experimental work, either on their clothing or from their person, as each person has a unique microbiome harbouring between 9 and 23 different fungal strains [30,31]. The wide variety of fungal counts observed between classrooms within each campus also reflects observations made of teaching spaces at other similar institutions. For example, average mould spore counts in the order of log 2–2.5/m^3 have been reported in teaching spaces where genera such as *Cladosporium* and *Aspergillus* have predominated [32,33]. While our results do not express mould counts in equivalent units, it is nevertheless interesting that other works' findings are based on data collected

from rooms within other institutions that also have a wide variability between classrooms (Log 1.0–4.8/m^3 for the examples given above). While the differences observed between the urban and semi-urban campuses are likely to have been influenced by the residential origins of the two different cohorts, it is acknowledged that the limited microbiological assessments carried out in this study are not sufficient to test this idea to a sufficient level of robustness. Nevertheless, the authors are of the view that genera such as *Aspergillus* likely play a role, as they have been reported as relatively ubiquitous in air samples collected across a range of seasonal variations [32]. In addition, the wide variances observed in both average mould counts and $PM_{2.5}$ values across study rooms limited the opportunities for making some statistical comparisons at the 95% confidence level.

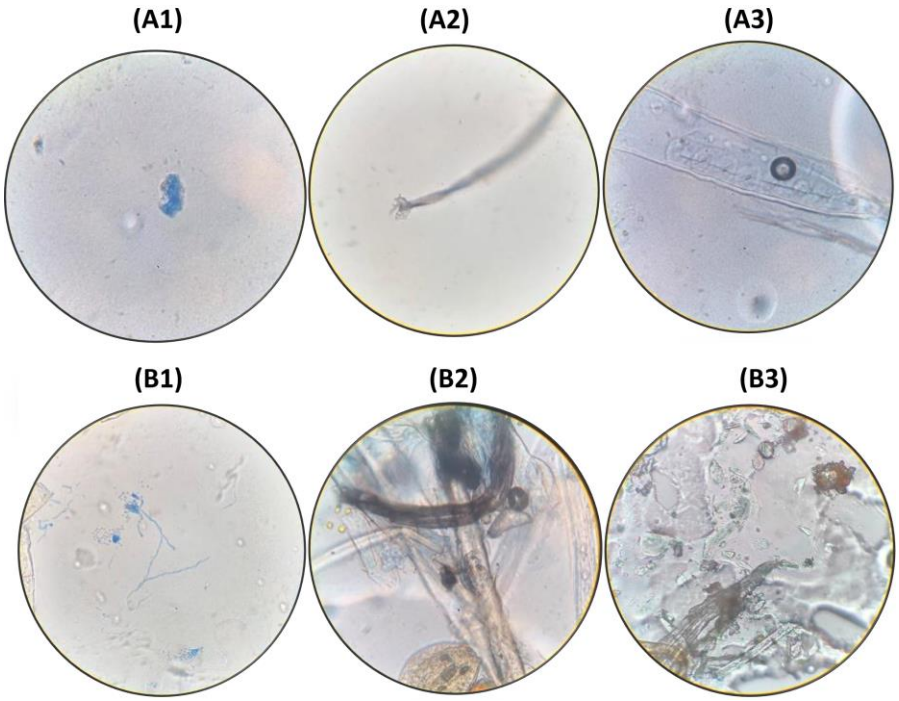

Figure 10. Examples of microscopic tape-test analyses of mould samples taken from the urban campus rooms (**A1**–**A3**) and semi-urban campus rooms (**B1**–**B3**). While all rooms indicated the presence of fungal hyphae, room B1 also indicated the presence of fungal spores.

Limitation of this study: There is no realistic way to eliminate mould growth in buildings because of the amount of spores produced and the manner in which they spread. The best approach is to limit indoor mould development by regulating moisture and providing appropriate air [7,33]. Fungi expected to be present in this study include *Stachybotrys*, *Cladosporium*, *Fusarium*, *Aspergillus* and *Rhodotorula*. However, it is acknowledged that room occupants may also bring in additional contaminants. While the settle-plate method has long been a standard method for enumerating such fungi, it is acknowledged that, as a passive method, settle-plate results reflect those spores that have settled out of the air. Hence, because settle-plates do not necessarily capture all of the spores suspended in a given body of air [28], our reported fungal counts may under-represent the numbers of viable spores captured by the more active air image sensor. It is therefore appreciated by the authors that a deeper study of the species involved is desirable, and a future study will therefore consider options for including active methods of fungi enumeration and relevant molecular analyses, such as rRNA sequencing, for such genera as *Alternaria*, which are known to be associated with asthma [34].

A further limitation of this study is the paucity of data points given the preliminary nature of this study, as well as limited information about the users of these classrooms. Given the wide variability observed in this study, a further study will include a larger range

of replicate rooms to strengthen statistical analyses. Future work will also include a survey of the rooms' users.

5. Conclusions

In this article, we evaluated the breathable atmospheres of classrooms with respect to mould and particulate matter in the winter period of the Waikato region of New Zealand. In this study, we illustrated the different characteristics of classrooms when they are located in urban and semi-urban areas. The mould counts in the urban classrooms tended to be lower than those in semi-urban classrooms with a difference of 63% higher at the Rotokauri campus. Furthermore, the results indicated that mould counts can be higher for newer buildings (semi-urban campus) compared with older buildings in the city centre (urban campus). These findings may be due to a range of factors. However, one factor at the semi-urban campus is the proximity to farming activities, which is commonplace in the Waikato rural area. The other finding is that the particulate matter in these classrooms responds to the outdoor $PM_{2.5}$ readings with a difference of 80% lower than that in urban classrooms (urban campus). These data indicate that when the outdoor PM values are close to or exceed the threshold of 25 $\mu g/m^3$, the classroom itself will be reading higher than the usual average of 2.5 $\mu g/m^3$. Furthermore, $PM_{2.5}$ values were found to be low for semi-urban rooms B2 and B3 compared with B1, which is a classroom in an old building experiencing mould and moisture issues compared with the other classrooms, as they have an advanced HVAC system with new technologies and filters (B2 and B3).

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