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Mind the gap between non-activated (non-aggressive) and activated (aggressive) indoor fungal testing: a short paper

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Abstract

Indoor fungal testing has been within the researchers' scope of interest for more than a century. Various sampling and analysis techniques have been developed over the years, but no testing protocol has been yet standardised and widely accepted by the research and practitioner communities. The enormous diversity in fungal taxa within buildings with varied biological properties, and implications on the health and wellbeing of the occupants and the building fabric complicates the decision-making process for selecting an appropriate testing protocol. This study aims to present a critical review of non-activated (or non-aggressive/passive) and activated (or aggressive/active) approaches focusing on the preparation of the indoor environment prior to sampling. The study emphasises the potential errors while interpreting results obtained from testing protocols based on non-activated and activated strategies.

Keywords: Mould; Fungal testing; Indoor fungi; Aggressive; Non-aggressive; Mould growth assessment

1. Introduction

Indoor fungal growth may affect the health of occupants (WHO, 2009; 2013), disturb their comfort and well-being, and lead to damage to the building fabric (Anderson, 1998; Singh, 1999). Therefore, it is of critical importance to be able to measure the extent of fungal growth correctly in a given indoor environment. Through testing protocols, researchers aim to quantify fungal biomass, determine the conditions under which fungi flourish, and assess whether the property needs remediation (Heinsohn 2007). However, though many protocols have been proposed and widely implemented, indoor fungal testing has not yet been standardised (BS EN ISO 16000-19:2014, Aktas, 2018a; Aktas et al, 2020).

Indoor fungal testing protocols are typically composed of the following steps: (1) the establishment of the environmental setting prior to sampling, (2) the collection of samples and (3) sample analysis via one or more techniques to estimate the amount and/or the contents of indoor fungal flora (Figure 1).

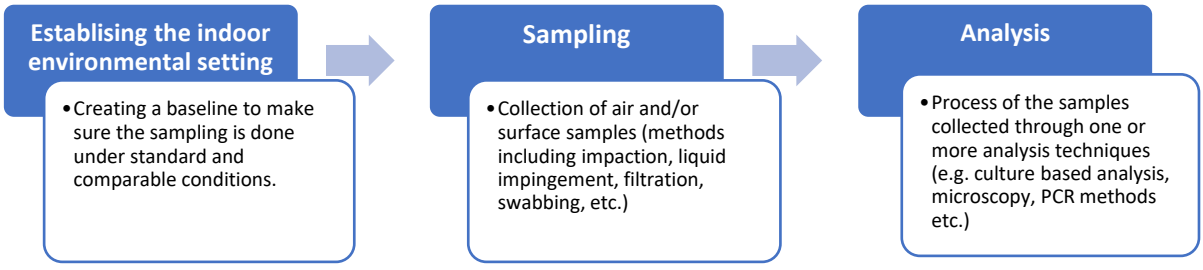

Figure 1: Schematic representation of the main stages of indoor fungal testing

The first stage of testing includes all the activities carried out prior to sampling i.e., the establishment of the indoor environmental setting. The preparation may be done by creating a completely still environment by restricting any access to or movements in the spaces to be tested for some time prior to testing (termed as non-

activated or non-aggressive sampling[1]) or by resuspending particles through mechanical means such as an air blower for a predetermined amount of time, often defined as a function of the room surface area or the volume (termed as activated or aggressive sampling). Between these two extremes are the protocols where the equipment setup and some movement of the occupants or the investigators prior to the testing are allowed (Table 1) (e.g. Gent et al, 2012; Dallongeville et al, 2015; Cahna et al. 2015; Aktas et al 2018a,b; Caballero et al 2021).

Examples of different protocols	*Exemplary studies*
(a) Waiting period where all movement is restricted — Equipment setup — SAMPLING	(Terčelj et al, 2011) | **Notes:** Windows and doors were closed for several hours prior to sampling. (Shinohara et al, 2021) | **Notes:** Tests were carried out in unoccupied spaces.
(b) Equipment setup — SAMPLING	(Gent et al, 2012, Dallongeville et al, 2015; Cahna et al. 2015; Caballero et al, 2021) | **Notes**: Activities prior to sampling were not restricted. The rooms were occupied before and during the sampling but no mechanical activation was carried out
(c) Equipment setup — Mechanical activation — SAMPLING	(Aktas et al 2018a,b) | **Notes:** Mechanical activation for approx. 1min/10m^2 was carried out after the equipment setup. Upon completion of the activation sampling was initiated.

Table 1: Some examples of non-activated (a & b) and activated protocols (c) [length of line segments not necessarily indicative of the durations associated to their labelled activities]

While there is substantial literature on the selection of the most appropriate combination of sampling and analysis techniques depending on the aim of the investigation (determination of the amount and/or the content of the fungal microbiota for reasons including moisture damage within the fabric or health concerns) and the availability of tools (Heinsohn, 2007), the standardization of protocols with regards to the preparation of the indoor environment prior to sampling has gained limited attention and interest (Buttner and Stetzenbach, 1993; Rao, Burge & Chang, 1996; Flannigan, 2011). This situation manifests as additional complexity when comparing and contrasting findings of multiple research studies, even when the same sampling and analysis techniques are used. This paper aims to fill this gap by detailing the impact of the indoor environmental setting prior to the sampling on the testing outcomes through a critical review of the literature (Section 2) and a series of laboratory experiments (Section 3) to discuss research and knowledge gaps, and implications on the practice (Section 4).

2. Non-activated (non-aggressive) and activated (aggressive) protocols

The non-activated (non-aggressive) and activated (aggressive) terms are often used to describe various air mixing conditions prior to sampling. However, the boundary between what is considered to be still or actively-mixed air is often unclear (Figure 2). Cahna et al., (2015) and Caballero et al., (2021) collected air samples for the assessment of the fungal contamination in classrooms while occupied by children. In another study by Shinohara et al., (2021), house dust samples were collected from unoccupied houses in Japan. Aktas et al, 2018b used a hand-held blower for 1 min per 10m^2 to resuspend particles prior to sampling. In all three cases, the environmental setting under which the air sampling was carried out was different. Hence, how they should be categorized is debatable – the findings are not comparable. Though the use of a blower might be best placed to reach the "saturation point" (Figure 2), where the other two cases sit within the wide spectrum of different levels of air stillness is still unclear. It should be underlined that this inability to fully understand how the different

[1] Please note that terms "passive" and "active" were previously proposed to indicate this differentiation, however as these terms are also used to indicate sedimentation and impaction, to avoid confusion the authors suggest non-activated (non-aggressive) and activated (aggressive).

protocols reflect on the readings has particularly strong implications for studies focused on health and the assessment of pathogenic and fabric-damage potential.

We therefore raise the following open research question:

What environmental settings pre-sampling ensure that the testing procedure is replicable, and lead to comparable results from different properties ?

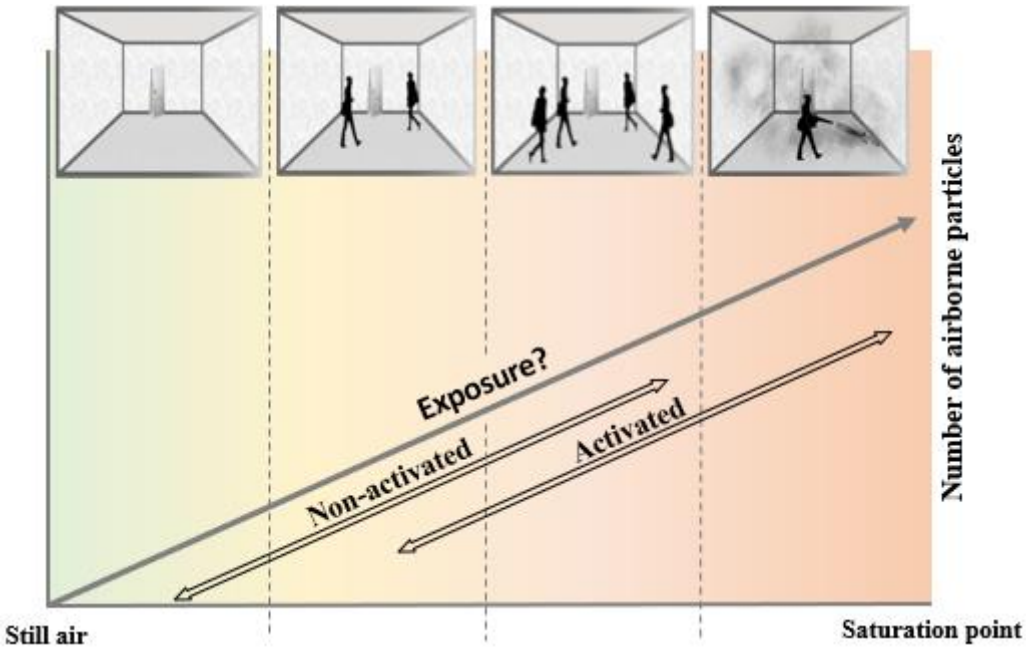

Figure 2: Illustration of non-activated (non-aggressive) and activated (aggressive) protocols

The right fungal testing protocol should ensure that in every building the **same** and **appropriate** potential indoor fungal reserves are assessed.

- ➔ The inspectors should be able to excite fungal particles from the **same** potential fungal reserves (Section 2.1) in every testing environment so that the testing outcomes reflect the fungal burden within a space without being affected by case-dependent variables

- ➔ The testing procedure should be able to assess the fungal growth from **appropriate** fungal reserves that are in line with the purposes of the investigation

Ensuring that the protocol performed in every property examines the **same** and **appropriate** reserves could minimize - if not eliminate - the effect of the different environmental conditions and inspector related biases (any arbitrary actions made before or during the sampling) to the readings and allow the capture of the fungal biomass that better represents the investigation targets. However, the development of such protocol might prove challenging unless the potential fungal reserves in a property are classified and the effect of the environmental conditions and activities on the resuspension of particles are fully understood.

2.1 Classification of potential fungal reserves

In the effort to understand how the same and appropriate potential fungal reserves in every property can be assessed, it is of utmost importance that the potential sources of fungi within a space are identified and classified. The indoor air contains aerosolised fungal particles that may originate from indoor fungal growth or the outdoor environment (Górny, 2004; Gutarowska et al., 2015; Yamamoto et al., 2015). If the biotic and abiotic factors are favourable for growth, fungi can grow in visible or hidden locations inside a property or may be stored or even grow in dust (Flannigan, 2011; Gent et al, 2012; Dallongeville et al, 2015; Nastasi et al, 2020; Andersen

et al, 2021). Therefore, the potential fungal reserves of particles within residential properties could be classified into four categories (Figure 3):

Category 1: Airborne fungal particles

Category 2: Visible locations including walls, floor, ceiling

Category 3: Hidden locations including walls behind furniture

Category 4: Locations which are difficult to be accessed including sinks, vents inside A/C units, behind internally insulated walls, inside the hollows of radiators

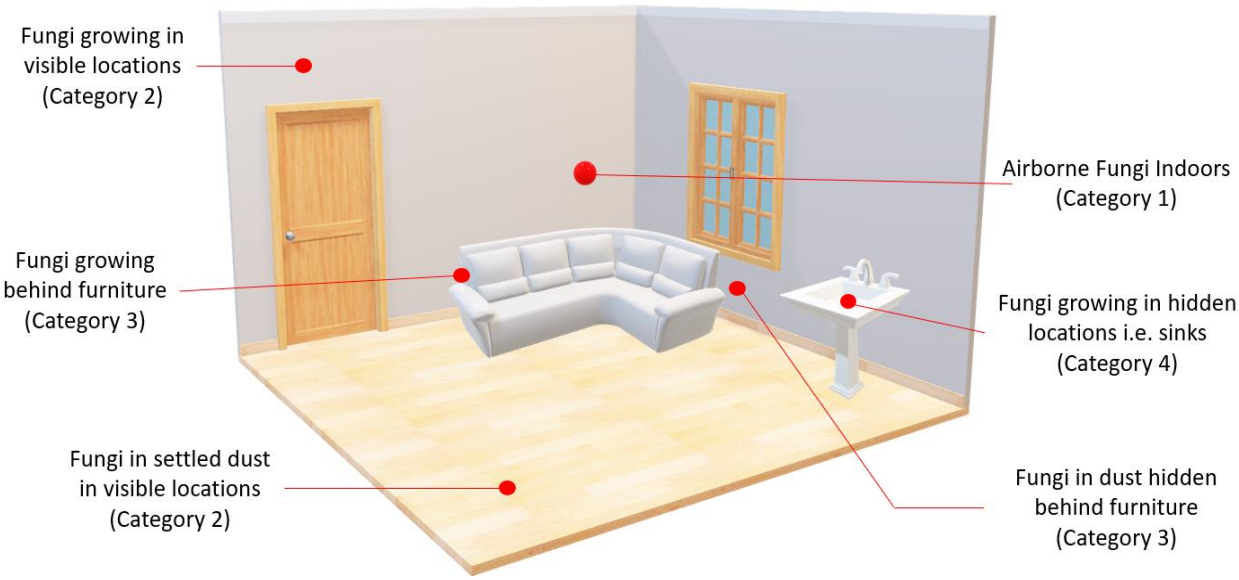

Figure 3: Locations where fungal reserves of particles can be potentially found within a property

Categories 1 and 2 include the indoor air and places easily accessible by occupants. Category 3 includes places that occupants rarely access, but movement or high level of activity close to these locations may lead to the resuspension of fungal particles. Lastly, category 4 includes locations inaccessible to occupants or rarely cleaned (e.g. corners behind heavy furniture, sinks, etc) and resuspension of particles from these locations is not expected. While assessing the three first categories is likely to allow the collection of samples that reflect the actual fungal biomass within a space, it is still unclear which reserves should be assessed for studies that focus on other targets, i.e. exposure.

2.2 Understanding the effect of activities and environmental context to the sampling readings

Many researchers have reported that the level and nature of the activities within a given indoor space do influence the resuspension of bioaerosols including the fungal ones (Flannigan, 1992 & 1997; Gots, Layton and Pirages, 2003; Heinsohn, 2007; Napoli, Marcotrigiano & Montagna, 2012; Rylander, 2015; Aktas et al., 2018b) (Table 2). The creation of artificial air currents due to the disturbance of the air's steadiness was found to increase the concentration of the airborne fungi by resuspending settled fungal matter, hence allowing the detection of particles that are otherwise not detectable (Rylander, 2015; Aktas et al., 2018b). However, the variations in the readings obtained from non-activated and activated protocols are yet to be fully understood. It is important to mention that the review of the literature indicates that the use of non-activated protocols is prevalent in indoor fungi testing-related studies (Zorman and Jersek, 2008; Gent et al., 2012; Adams et al., 2013; Dallongeville et al., 2015). Nevertheless, though some researchers recommend that non-activated protocols should be selected over activated ones under the claim that the collection of samples from still air can reduce reproducibility-related errors (e.g. Heinsohn, 2007), other studies using both protocols comparatively suggest otherwise (e.g., Aktas et al., 2018a,b). Mukai et al., (2009) suggest that increasing the level of the activities prior to testing is expected to reduce the differences in resuspension rates of particles compared to when lighter or no activity is carried out prior to testing.

Research by	Context/ Sampling and analysis method:	*Outcomes*
Flannigan (1992)	Field work/Impaction and colony enumeration	Suggested that during the occupied hours, the human activity led to a significant increase in the resuspension of fungal particles in the restaurant tested leading to an increase of the viable fungal counts (Approximately 16 times higher than the readings during unoccupied hours)
Rylander, (2015)	Field work/Filtration and NAHA measurements	Reported that that the indoor fungal levels (NAHA levels) in rooms in villas and apartments with no mechanical ventilation increased up to 10 times when an activated protocol using a blower was implemented compared to the readings when a non-activated protocol was selected.
Aktas et al, (2018b)	Field work/Impaction on Agar plates and colony enumeration & Filtration and NAHA detection	Reported an up to 56 times increase of the activated fungal readings compared to non-activated readings when testing was carried out in residential buildings in North London
Mundt, (2000)	Laboratory experiments /Particle counters used to measure airborne particle concentrations	Indicated that the increase of the indoor air velocity will increase the resuspension rate of particles
Mukai et al, (2009)	Laboratory experiments /Particle counters used to measure airborne particle concentrations	Indicated that the increase of the indoor air velocity will increase the resuspension rate of particles
Goldasteh et al, (2010)	Laboratory experiments /Particle counters used to measure airborne particle concentrations	Reported 1) approximately 10 times higher resuspension rates of 1-10μm particle from hardwood flooring and 2) approximately 2-3 times higher resuspension rate of 1-10μm from linoleum flooring when airflow speed increased from 4.5 to 21 m/s in a laminar flow wind tunnel
Napoli, Marcotrigiano & Montagna, (2012)	Field work/Impaction and total viable counts (TVC enumeration)	Reported an increase of the mean TVC by approximately 7.5 times during operations in hospital rooms in the Apulia Region in South-eastern Italy.
Wang et al, (2021)	Field work/ Particle counters used to measure airborne particle concentrations	Reported approximately a 5.5 times increase of average PM2.5 concentration when the occupancy density increased from 10 to 35 people in a classroom of Jinnan Campus of Nankai University

Table 2: Effect of the activation to the fungal readings reported in the literature.

An important concern regarding the detectability of fungal particles, especially the larger ones, is how quickly the airborne fungal levels return to the pre-activation levels. Different activities may lead to different levels of particle resuspension. Allowing a long period of time to pass after the end of the protocol may lead to the settlement of heavy particles before they are able to be collected through sampling (Tucker et al., 2007). Mundt (2000) suggests that large particles are expected to settle faster than small ones after indoor activities have stopped, as also supported by our findings (see Section 4). To that end, the inability to specify the duration of time needed between the preparation of the indoor environment and the sampling, might lead to variations in the sampling results.

Changes in the **pattern and the speed of the inspector's movement** across spaces may lead to the resuspension of particles from different locations, potentially contaminated by fungi. This could raise comparability issues for the data collected from space to space. However, addressing this issue becomes particularly important in cases where non-activated protocols are adopted. Findings by Rylander (2015) indicate that the movement of inspectors inside residential buildings prior to testing increased the measured concentration of fungal biota by 2 times compared to when all movement was strictly prohibited. Small scale experiments and Computational Fluid Dynamics (CFD) tests carried out by Cao et al., (2017) indicated that human movement within a room could affect the dispersion of particles and that increase in the movement's velocity is likely to lead to longer resuspension of fine particles (0.02-1μm).

The effect of the **room characteristics/condition (cleanliness, level of upholstery etc.) and furniture arrangement on the readings** to the sampling readings is not fully understood. The cleanliness levels can be connected to the amount of dust within a property and abiotic factors such as water and nutrient availability

affecting the sporulation of certain fungal species (Buttner and Stetzenbach, 1993; Aktas et al, 2018). In different properties, the quantity of dust and the fungi stored in it can vary. The volatility of fungi in dust can also change (Metz et al., 1979). Hence, the particle aerosolization rates may vary due to the different dust levels and volatility of fungal particles in every space and thus potential underestimation/overestimation issues regarding fungal readings may be risen.

Another concern with regards to both the comparability of the testing outcomes from the current testing procedures and their ability to assess the appropriate potential fungal reserves is the uncertain effects of the **hygrothermal conditions** on the readings. The relative humidity affects the fungal growth and sporulation (Arthurs and Thomas, 2001; Zhao and Shamoun, 2006; Money et al, 2016) directly, but can also indirectly influence the resuspension rates and the recovery efficiency of fungal particles indirectly. Depending on the fungal species present the optimal relative humidity is likely to increase the spore production rate and conidial growth (Arthurs 2001; Zhao 2006; Money et al, 2016). However, the moisture content in the indoor environment and the indoor materials can also affect the adhesion of fungi on the indoor surfaces and may vary across different spaces (Osherov & May, 2001). In high humidity conditions, liquid molecules can be adsorbed on small-sized bioaerosols, including small-sized (>0.1μm) fungal particles leading to an increase in the adhesion forces between the particles and the surfaces they come from in contact with (Baron and Kulkarni, 2011). As a result, the aerosolization rate of particles by implementing the testing methods may vary from case to case, thus influencing the sampling readings.

3. Experimental Work

In order to investigate on a quantitative basis, the impact of the level of "activation" on the obtained readings, an experimental campaign was designed, and three sets of experiments were carried out. The experimental work aimed to identify the role of the blowing duration in the particle counts at different heights. The findings of this experimental work are then discussed in conjunction with the literature. For these experiments particle counter readings were chosen as a proxy for indoor mould testing outcomes. This was considered a robust approach since fungal particles can range in size from 0.6μm – 10μm (Claub, 2015) and associations between the airborne fungal concentration changes and particle counts have been previously reported in the literature (Agranovski et al., 2004; Brandl et al., 2008), the examination of the activation's effect to the sampling readings can be further explored if the literature search is expanded to incorporate research documents that have studied the effect of the indoor airflow patterns to the resuspension of non-fungi specific particles of different size too.

The work was conducted in an environmental chamber (2.8x3x3m) where particle counters were positioned at three different heights - the readings from 6 particle size channels were analysed before and after activation (Figure 4). To ensure that dust or small particles cannot infiltrate the room through cracks and openings, the environmental chamber joints were entirely air-sealed with sealing tape typically used to perform blow-door tests. It is important to mention that the ventilation system in the chamber was not operated at any instance during these tests, and the ducts leading to the chamber were also sealed to avoid unintended disturbance in the air.

Figure 4: Experimental setup inside the environmental chamber.

Three particle counters (PMS 5003 by Plantower, Sensitivity: 50% - 0.3 µm, 98% - 0.5 µm and larger, Resolution: 1 µg/m³, work temperature: od -10 °C do 60 °C, Humidity (work): 0-99%) were placed at heights of 0.75m, 1.5m and 2.25m on a vertical pole located in the centre of the room to monitor the variation of different sized particles with height. All three sensors were calibrated by the manufacturer prior to use, and were operated remotely.

To implement activated protocols within the environmental chamber, a leaf blower (MODEL 100760 Merry Tools Air Leaf Dust Blower Electric Inflator) was used. The blower was placed on top of a Bluetooth operated slider (Neewer Motorized Camera Slider) and was connected to a wifi-enabled plug to ensure that it could be operated remotely. The blower was set at a fixed distance from the wall (distance between nozzle end and wall =1.5m) and was able to move horizontally for a distance of 1m (Figure 5).

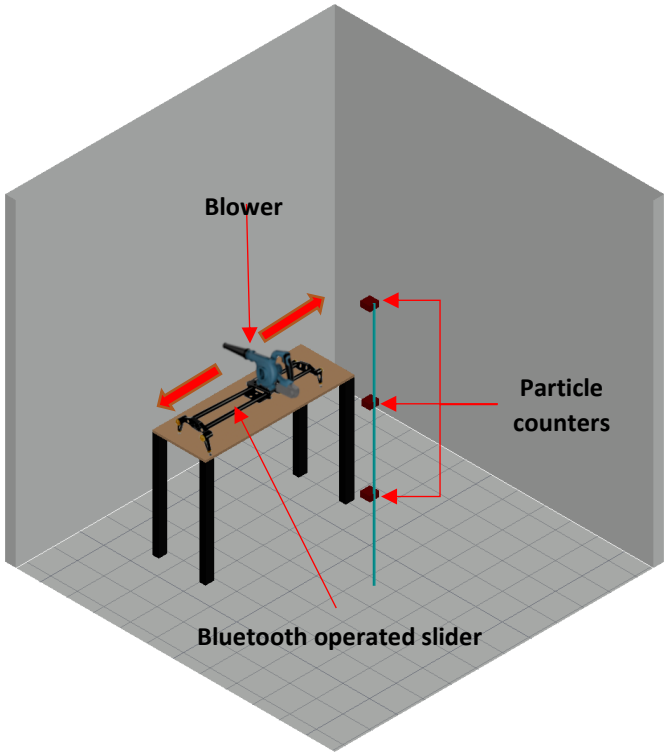

Figure 5: Schematic representation of the experimental setup and the movement of the blower inside the environmental chamber.

Experiments were conducted to examine the effect of 6 different blowing durations on the concentration of particles at the three different heights in the centre of the room. Tests were repeated three times (Series 1, 2, and 3 in Figure 6) to reduce the likelihood of anomalous results and increase the accuracy of the experimental outcomes. The blowing durations were set to be 1min, 2min, 3min, 5min, 10min and 15min. During these periods, the blower was set to move horizontally from one end of the slider to the other, while blowing towards the wall at a rate of 3.5 m³/min.

The particle counters were set up to start logging measurements 1 hour before the start of the blowing and continued until 8 hours after it. It is important to mention that the time interval between particle logs was set to be 1 minute and the logs represent the average number of particles counted every second (~1 reading/sec). This approach was selected to maximize the input data and prevent any unwanted errors due to potential failure of data transmission through the serial communication.

4. Results and Discussion

The outcomes of the experimental work are plotted in Figure 6. Results indicate that the particle resuspension rates increase with the increase of the blowing time for all three sets of experiments. Of particular interest is that for blowing durations of more than 5min, the smaller particles (Particle size < PM1) did not return to the levels before the blowing was initiated, **even after 7 hours**. However, the same cannot be stated for larger

particles. For the same blowing duration, the pattern of the particle settlement becomes more unclear with the increase in the aerodynamic diameter (AD).

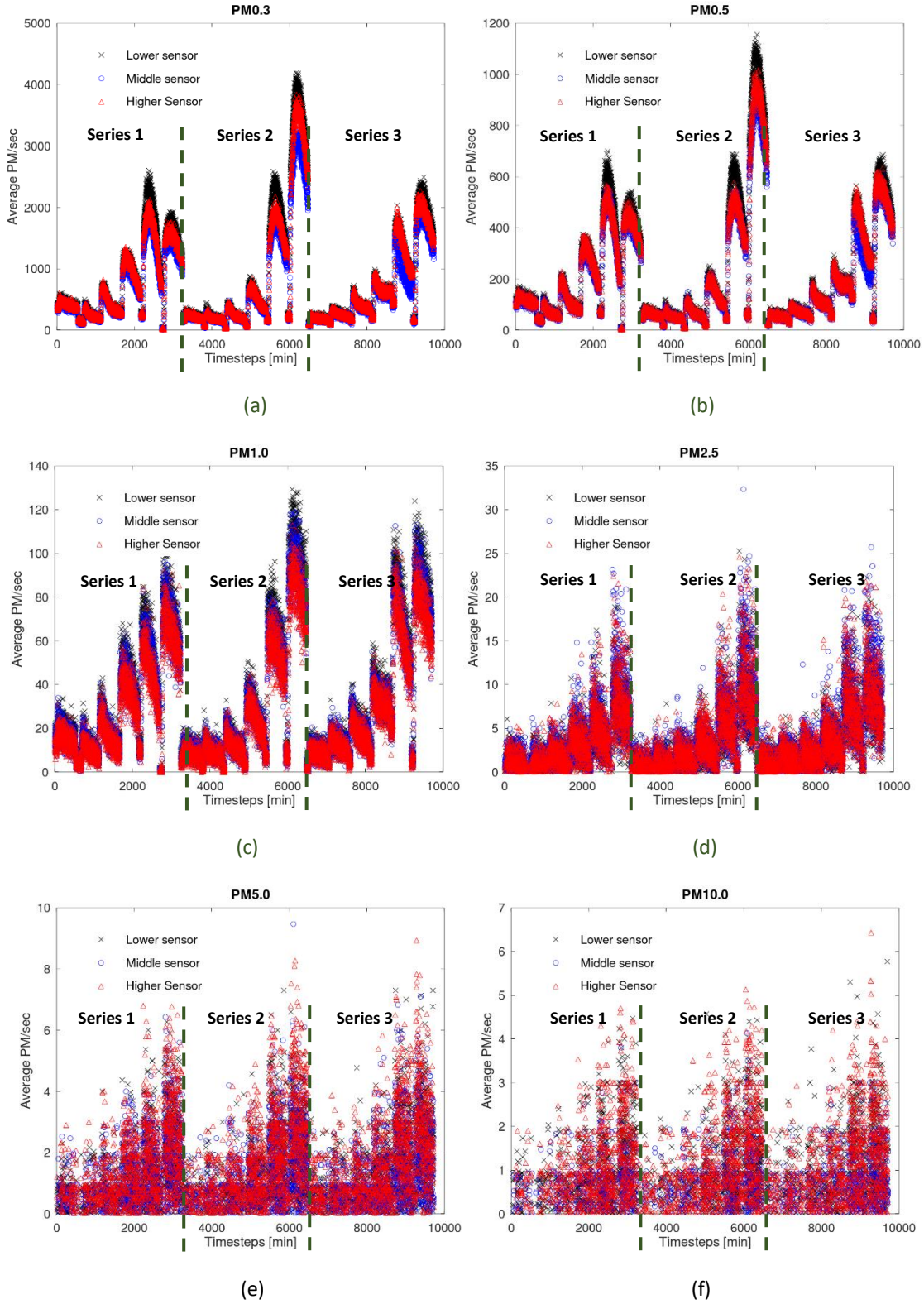

Figure 6: Particle counts for (a) PM0.3, (b) PM0.5, (c) PM1.0, (d) PM2.5, (e) PM5.0, and (f) PM10 from all the experiments conducted

To better understanding of the pattern of the particle readings with the increase of the blowing duration, the average number of the PM0.3, PM2.5 and PM10 over 1minute intervals were plotted against time for three

cases of blowing duration including 1min, 5min and 15min (Figure 7). The higher blowing durations have led to higher resuspension rates for all three size particles at any height.

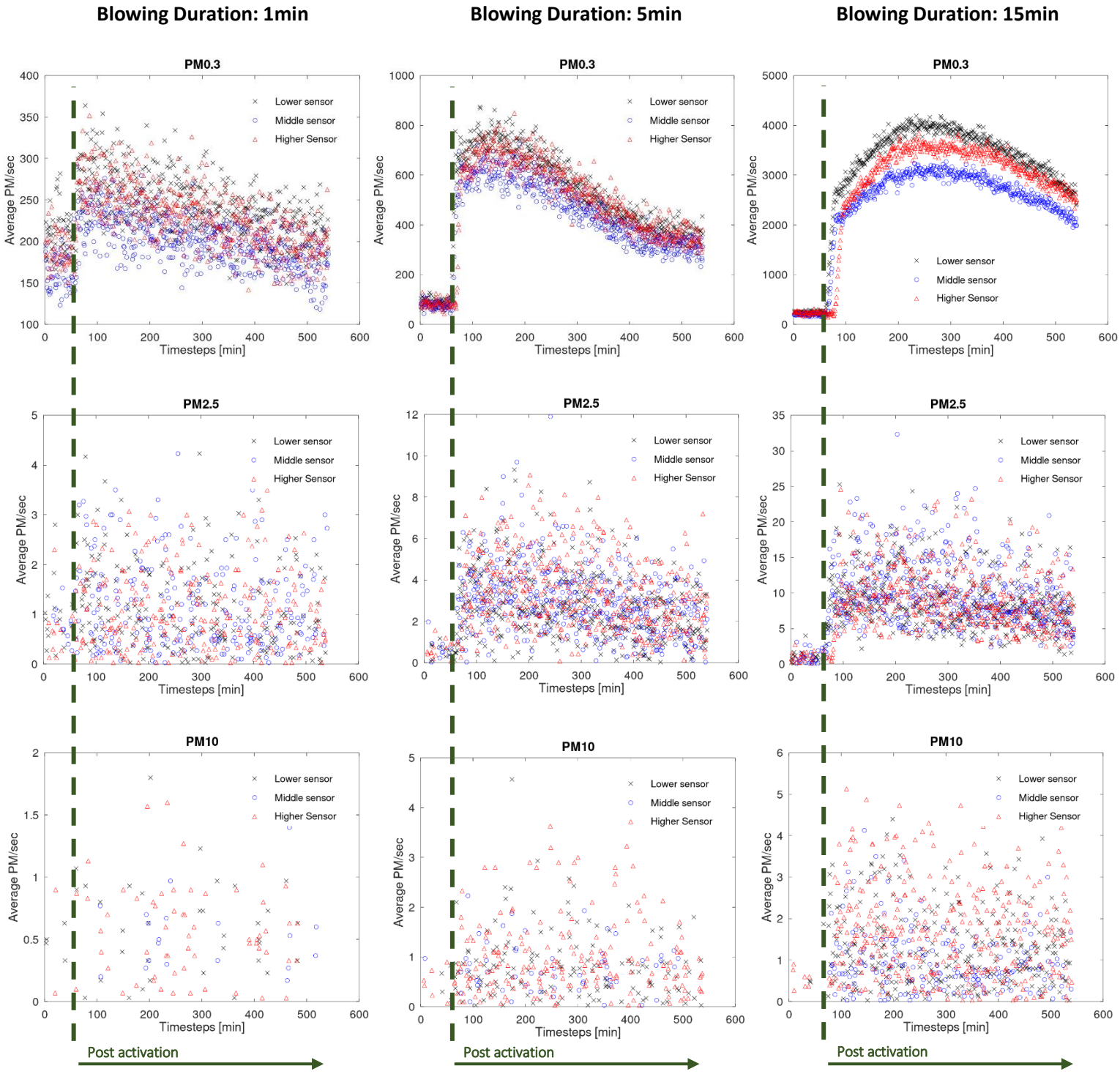

Figure 7: Measurements of PM0.3, PM2.5 and PM10 at different heights for 3 blowing durations (1min, 5min, and 15min)

The number of different sized particles measured at different heights was linearly projected to detect the effect of the blowing duration on the particle counts with height (Figure 8). The increase in the blowing duration affected the resuspension rate of both small and large particles. The difference in the particle numbers due to the change of the blowing duration is better captured by the lower particle sensor than the middle and higher one (Figure 8(a)). However, Figure 8(c-d) shows a more uniform increase in the levels of the particles when their

AD is larger than 1µm with an increase in the blowing duration compared to the levels of PM0.3 and PM0.5 Figure 8 (a-b). Higher levels of the small particles (PM0.3 and PM0.5) were measured by the lower sensor than the higher one.

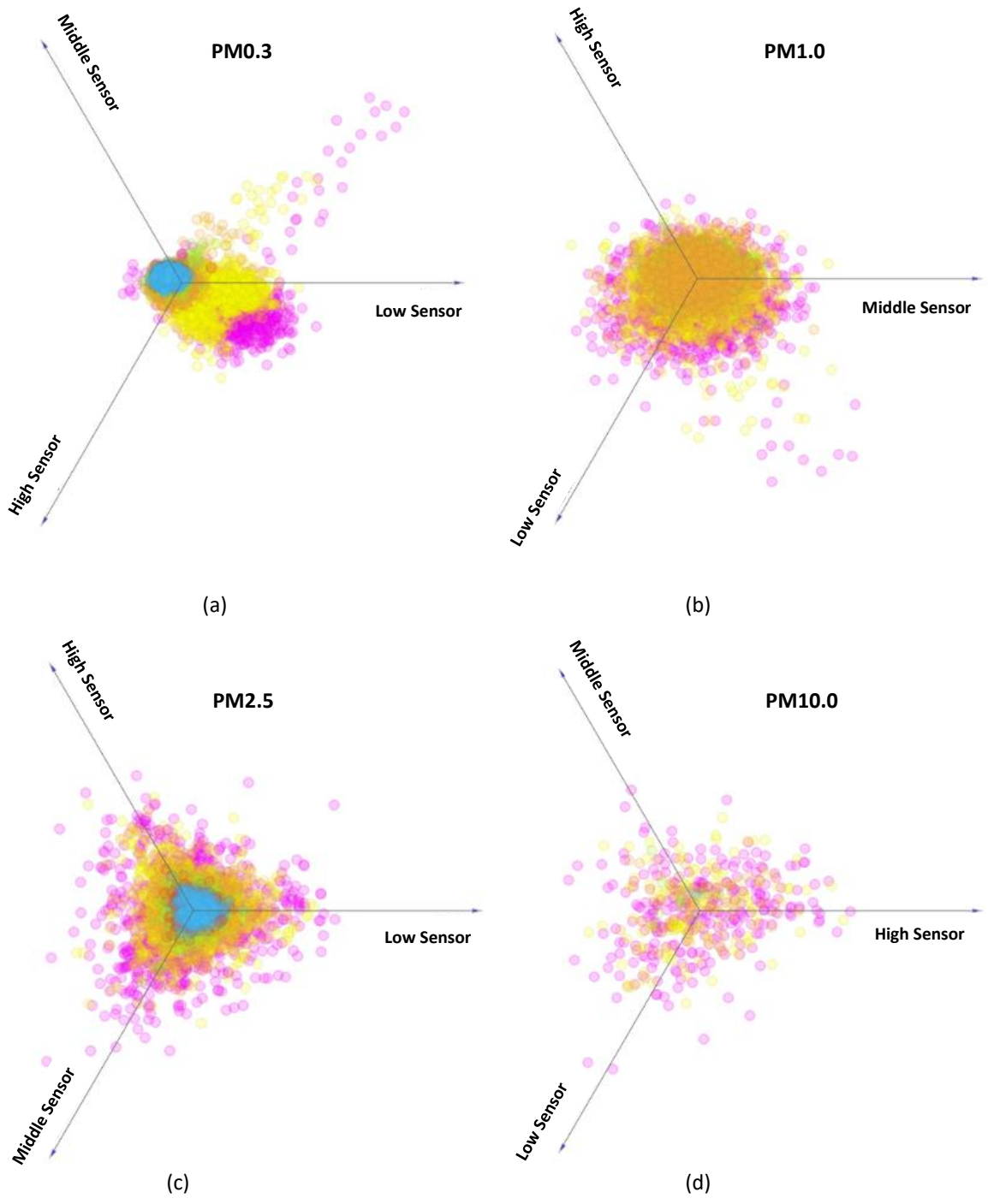

Figure 8: Linear projection of same-sized particle readings measured at different heights

The maximum number of particles measured at each height in every experiment were plotted against the blowing duration and are demonstrated in Figure 9(a-f). The coefficients of determination [R^2] for all three series suggest that the maximum number of particles of all sizes is strongly correlated to the blowing duration with the PM1.0 and PM2.5. In conjunction with Figures 6 and 7, the increasing trend of the maximum values of particles with the prolongation of the blowing duration suggests that the increase of the activities duration prior to sampling leads to higher particle resuspension rates.

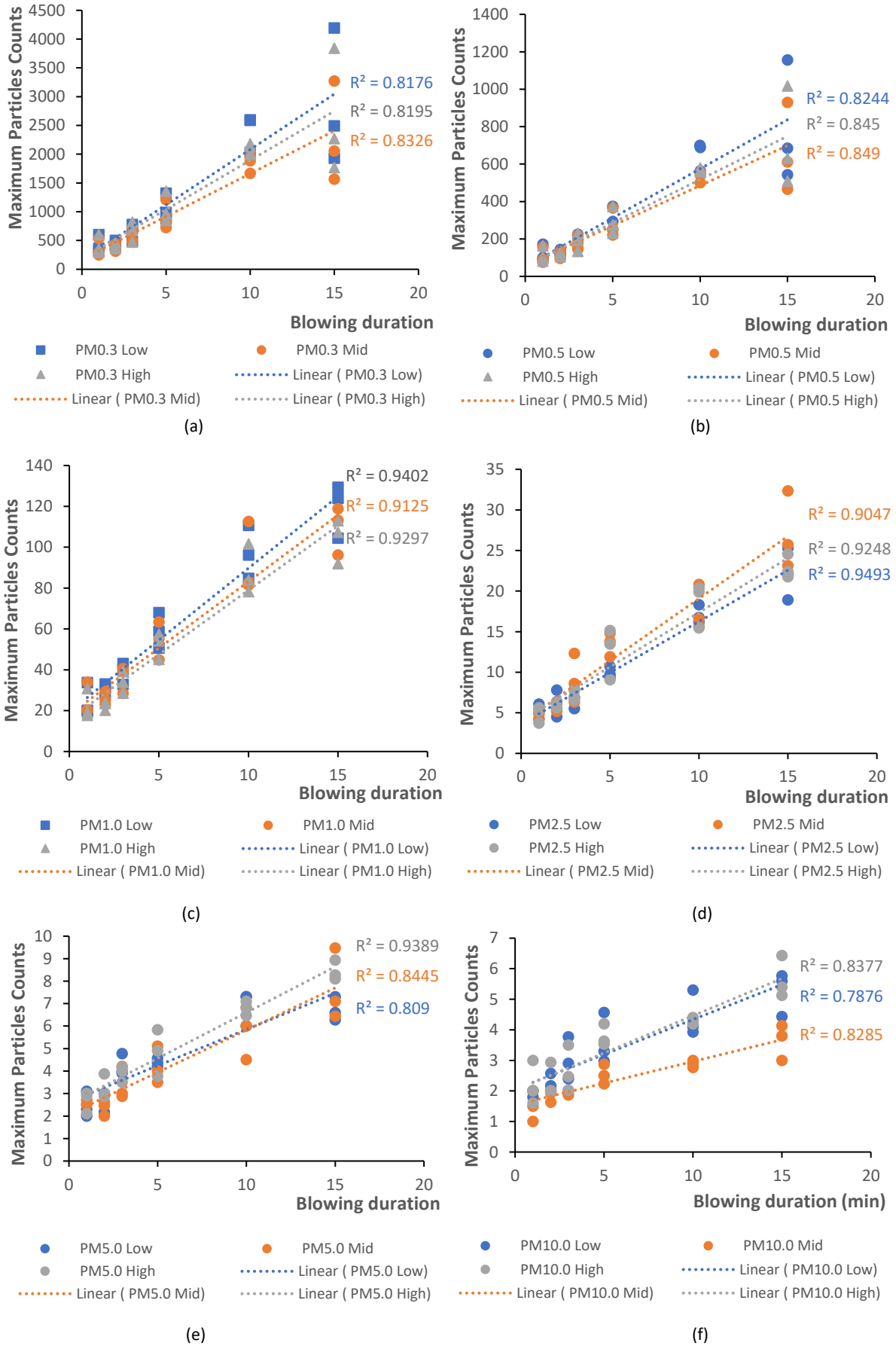

Figure 9: Maximum levels of different sized particles with height for the three experimental series

The Pearson's correlation coefficient was calculated to identify potential correlations between the number of different sized particles and the height, and a correlation matrix was created (Table 3). The results of the analysis indicate that the small particles (PM0.3 – PM2.5) correlate strongly with each other regardless of the height of the sensor (R>0.6). On the other hand, no strong correlations between the larger particles (PM5 and PM10) were identified with the highest value of R being 0.215 for PM5 particles measured by the higher and lower sensor and the lower R-value being the one for PM10 particles measured by the lower and higher sensor (R=0.058). It should be noted that in a previous study by Luoma and Batterman (2001), no significant variation was reported between readings of PM1 or smaller particles with height (particle counters placed at 0.4, 1.1, 1.8 m). On the other hand, the readings of particles ranging from PM5.0 – PM25 were shown to vary significantly with height. (Luoma and Batterman, 2001)

	PM0.3 Low Sensor	*PM0.3 Mid Sensor*	*PM0.3 High Sensor*
PM0.3 Low Sensor	1.000		
PM0.3 Mid Sensor	0.993	1.000	
PM0.3 High Sensor	0.986	0.986	1.000
	PM0.5 Low Sensor	*PM0.5 Mid Sensor*	*PM0.5 High Sensor*
PM0.5 Low Sensor	1.000		
PM0.5 Mid Sensor	0.993	1.000	
PM0.5 High Sensor	0.985	0.986	1.000
	PM1.0 Low Sensor	*PM1.0 Mid Sensor*	*PM1.0 High Sensor*
PM1.0 Low Sensor	1.000		
PM1.0 Mid Sensor	0.964	1.000	
PM1.0 High Sensor	0.951	0.953	1.000
	PM2.5 Low Sensor	*PM2.5 Mid Sensor*	*PM2.5 High Sensor*
PM2.5 Low Sensor	1.000		
PM2.5 Mid Sensor	0.606	1.000	
PM2.5 High Sensor	0.606	0.611	1.000
	PM5.0 Low Sensor	*PM5.0 Mid Sensor*	*PM5.0 High Sensor*
PM5.0 Low Sensor	1.000		
PM5.0 Mid Sensor	0.142	1.000	
PM5.0 High Sensor	0.215	0.211	1.000
	PM10 Low Sensor	*PM10 Mid Sensor*	*PM10 High Sensor*
PM10 Low Sensor	1.000		
PM10 Mid Sensor	0.096	1.000	
PM10 High Sensor	0.058	0.090	1.000

Table 3: Correlation coefficient for same-sized particles measured at different heights

The Levene test was used to examine the equality of the variance for the number of same sized particles measured by the same sensor during all three experiments. For a significance level p_{value}=0.05 the tests have indicated no homogeneity of the variances between the three experimental series for all cases tested. This suggests that though the tests were repeated in the exact same way the variances of the readings are significantly different. These differences might be a result of the limited horizontal movement of the blower and the limited area being directly affected by the high air-velocity output. Nevertheless, this conforms with the literature outcomes in section 2 and adds extra value to the statement regarding the comparability concerns due to limited control over the activities and the testing conditions prior to sampling.

To understand the extent of the activation's effect on the particle readings, the differences between the maximum and minimum particle counts captured by every sensor were averaged for all experiments carried out for 1min and 15min blowing durations and plotted against particle size in Figure 10. The blowing duration has led to an increase in the particle count variation for all particle sizes – with the difference being the largest for the smaller particles (> 4000 particles for PM0.3 and 15min blowing) and for PM10 particles (<7 particles) and blowing duration of 15min. This can indicate that the readings for the PM1.0 or smaller particles capture the

blowing duration changes easier than the larger particle counts. Therefore, monitoring the small-sized particles (<PM1.0) could be a better proxy of the intensity of the activities carried out prior to sampling. It is important to mention that despite the prolonged blowing duration (15min), the variation in the large-particle readings (>PM2.5) was only slightly larger than the corresponding one when the blowing duration was 1min, indicating that the air activation might be more critical for the recovery efficiency of PM2.5 and larger particles during the sampling.

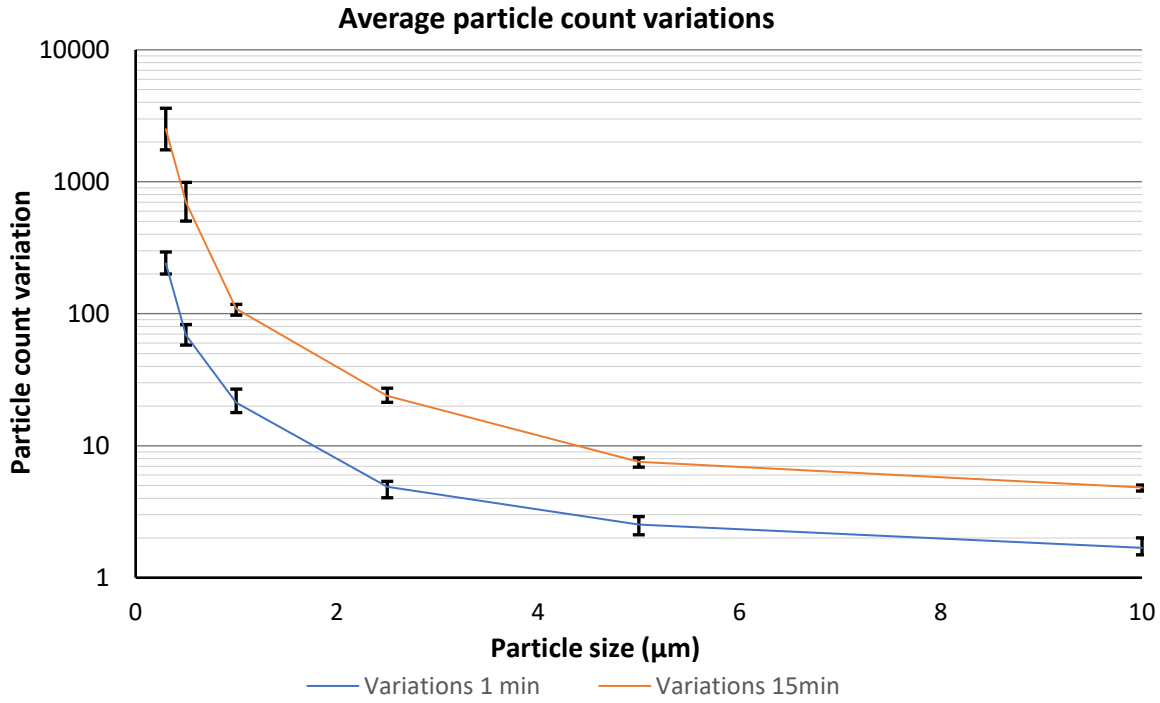

Figure 10: Minimum and maximum readings' variations for 1min and 15min blowing respectively

Discussion

The experimental work has shown that the particle readings, regardless of the particle size, follow an increasing trend with the increase of the blowing duration. However, the impact of air activation on larger particle readings (>PM2.5) was considerably weaker than the effect of the smaller ones (<PM2.5). Previous studies (Quian and Ferro, 2008; Wang et al., 2021) have shown that the indoor air velocity close to a dust reserve and the activation method used can affect the particle resuspension rates. Still, they have also stressed that large particles might be more inert to activation due to their size and the strong adhesion bonds between them and the surfaces they are attached to. Therefore, investigators should consider that a strict-non-activated protocol might seriously underestimate the particle intensity with important implications in relation to the indoor fungal levels and the health impact.

The particle counters' low sensitivity with PM0.3 readings (50% sensitivity) indicates that the readings of this channel might not be an accurate proxy for the evaluation of the indoor activities before sampling. Still, they might be able to show a trend for the PM0.3 concentration. Given also that it is highly probable that the majority of fungal particles will range between 1.0 µm AD and 3.2 µm AD (Claub, 2015), it can be stated that the PM0.3 readings would not be a suitable metric for the indoor fungal resuspension rates. On the other hand, the high sensitivity (>98%) of the sensor for particles larger than PM0.5 and the considerably higher particle count differences for smaller-sized particles (<PM1.0) with the increase of the blowing duration (Figure 10) indicates that the PM0.5 and PM1.0 readings reported here could be the most appropriate indicators of the activities carried out before sampling.

The high correlation coefficients between readings obtained at different heights for PM0.5 and PM1.0 indicate that the height at which the sensors are placed will not affect their ability to capture the concentration changes

of small particles. This conforms with previous studies showing that regardless of the sensor's height, unlike the coarser particles (>PM5.0), the smaller airborne particle counts will follow similar trends due to their ability to spread throughout an indoor space unrestricted of their size (Montoya & Hildemann, 2005). However, the same cannot be stated for larger particles (>PM2.5) where the low correlation coefficients suggest that the height of the sensor had led to noticeable differences in the particle count changes with time.

5. Conclusions

Researchers should give attention to the conditions under which the testing is carried out. Non-activated and activated readings do differ, therefore indoor fungal levels cannot be benchmarked unless uniformity is brought to the pre-sampling conditions through a well-established testing protocol. In the case of activated sampling, disturbing the air's stillness may increase the concentration of airborne fungal fragments and spores and thus could lead to the identification of fungi that could otherwise be undetectable. The experimental work carried out in the context of this study suggests that the use of a blower and the increase of the blowing duration could lead to higher particle resuspension which in real case scenarios could lead to higher detectability of particles ranging from 0.3-10μm. It should be underlined that the findings suggest that the smaller particle (PM0.3 to PM1.0) readings may be able to capture the different levels of activity better than the readings from larger particles (PM2.5 and PM5.0). In addition, the experimental outcomes showed that the increase of the larger particles with the increase of the blowing duration is noticeably lower than the one observed for the smaller ones. Therefore, the selection of an activated protocol prior to sampling could be of critical importance for the increase of the large particles collection efficiency. In any case, the extent of the activation's effect to the sampling readings should be further examined, and future works should focus on how the non-activated/activated protocol manifest in the sampling readings and how these can reflect the investigation aims.

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