A pilot investigation into associations between indoor airborne fungal and non-biological particle concentrations in residential houses in Brisbane, Australia.

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Abstract

Indoor air contains a complex mixture of bioaerosols such as fungi, bacteria and allergens, as well as non-biological particles including products from various combustion processes. To date little work has been done to investigate interactions and associations between particles of biological and non-biological origin, however, any occurring interactions could affect pollutant behaviour in the air and ultimately the effect they have on health. The aim of this work was to examine associations between the concentration levels of airborne particles and fungi measured in 14 residential suburban houses in Brisbane. The study showed that no statistically significant associations between the fungal spore and submicrometre particle concentrations or $PM_{2.5}$ were present, while a weak but statistically significant relationship was found between fungal and supermicrometre particle concentrations. A similarity in behavior between the submicrometre particle and fungal spore concentrations was that the fungal spore concentrations were related directly to the distance from the source (a nearby park), in a very similar way in which the submicrometre particles originating from vehicle emissions from a road, were dependent on the distance to the road. Recommendations have been provided as to the future study designs to gain a deeper insight into the relationships between biological and non-biological particles.

Keywords: airborne fungi, submicrometre particles, supermicrometre particles

Introduction

Recent epidemiological studies in several countries have indicated an association between human exposure to fungal spores in indoor air and adverse respiratory symptoms (Verhoeff and Burge, 1997). Other studies provided evidence that exposure to airborne particles, specifically the smaller ones, contributes to excess mortality and morbidity (eg Dockery et al. 1993). Elevated levels of particle air

pollution have been associated with decreased lung function, increased respiratory symptoms such as cough, shortness of breath, wheezing and asthma attacks, as well as chronic obstructive pulmonary disease, cardiovascular diseases and lung cancer. Indoor air contains a complex mixture of bioaerosols such as fungi, bacteria and allergens, as well as non-biological particles including tobacco smoke, cooking-generated particles, motor vehicle exhaust particles and organic and inorganic gases (CO, SO_2, NO_2, O_3). To date little work has been done to investigate interactions and associations between particles of biological and non-biological origin, instead, physical, chemical and biological properties of particles have most often been studied in isolation. However, it is considered probable that interactions may occur between biological and non-biological pollutants that could affect their behaviour in indoor air and ultimately the effect they have on health. There is a possibility that secondary dispersal of fungal allergens on the surface of smaller particles or spore fragments occurs as a result of an attachment process. Two studies have indicated interactions between particles and fungi (Glikson et al., 1995, Ormstad et al., 1998). Vehicle exhaust particles were examined as allergen carriers and cat allergen was found on the surface of the dust particles (Ormstad et al., 1998). In a study of outdoor air components, cytoplasmic content of spores and pollen was often found attached to vehicle exhaust particles (Glikson et al., 1995). Therefore non-biological particles may be serving as carriers of fungal allergen molecules into the lung independently of the whole fungal spore. In the case of non-viable combustion particles such as tobacco smoke or cooking-generated particles (<1 μm), such an interaction would have serious implications, as allergen molecules could conceivably be carried deeper into the lung than a fungal spore would be expected to penetrate. At the very least, the aerodynamic behavior of a coupled particle within the lungs may be expected to be quite different from that of two non-associated particles. Therefore, it is of critical importance to determine whether associations occur between non-biological

and biological aerosol particles, and if so what the nature of interactions causing the associations might be.

Non-biological particles that are airborne are generated by a multiplicity of sources. Fine particles (smaller than 2.5 μm) are generated mainly from combustion and photochemical processes and gas to particle conversion. The vast majority of these particles are in the ultra fine range. Coarse particles are generated from mechanical processes including grinding, breaking and wear of material and dust re-suspension. Air quality legislation in the U.S.A. has recently (1997) defined separate standards for mass concentration of non-biological particles less than 2.5 μm in diameter, following findings that exposure to these particles causes adverse health effects.

The majority of the indoor airborne fungal population is derived from outdoor sources, in particular from regional vegetation, which is known to strongly affect the nearby airborne fungal concentrations. However, when suitable conditions are present (relative humidity, temperature, air exchange rate) fungi may also flourish on indoor man-made structures. The deposition of fungal spores in the lung and their effect on human health depends on their composition (genera and species), concentration, and size (Reponen et al., 1996). While larger spores (>10μm) are deposited in the upper airway (nose, pharynx) and may result in hay fever symptoms, smaller spore particles (diameter <10μm, especially <5μm) can penetrate the lower airways and may lead to allergies or asthma (Horner et al., 1995). Unattached or attached fungal allergens that are in the ultra fine range (<0.1μm) or submicrometre size, respectively, can penetrate to the deepest parts of the respiratory tract. Fungal allergens are proteins of 10,000 to 50,000 Dalton occurring on the surface of the fungal spore.

A large number of studies have linked exposure to airborne fungi with various health effects. For example, an indoor air study in Finland found that exposure to airborne indoor fungi increased the risk for respiratory symptoms and infections by a factor of two (Nevalainen, 1999). Although the mechanisms involved are not totally understood, they include allergic, infectious and toxic reactions. More than eighty genera of fungi have been associated with symptoms of respiratory tract allergies (Horner et al., 1995). *Cladosporium, Alternaria, Aspergillus,* and *Fusarium* are amongst the most common allergenic genera. Metabolites of fungi including toxins and volatile organic compounds are also thought to irritate the respiratory system. In a study examining fungi and home factors in Kansas (U.S.A.), elevated concentrations of *Cladosporium* were frequently associated with respiratory symptoms (Su et al., 1992). Similarly, a comparison study of allergic and non-allergic children in Taiwan found that higher concentrations of *Cladosporium* and *Penicillium* existed in the homes of the allergic children (Li et al., 1995).

The work presented here is a part of a larger study focused primarily on fine and ultra-fine particles in indoor air and on outdoor to indoor penetration of particles in residential suburban houses in Brisbane, for different building types and ventilation characteristics (Morawska et al 2001). Number distributions and concentrations of all airborne particles in fourteen houses were measured in the size range from 0.015 to 20 μm, as well as approximation of $PM_{2.5}$ fraction (mass concentration of particles with aerodynamic diameter smaller that 2.5 μm). Biological particles investigated included concentrations of airborne fungi and concentrations of bacteria and allergen (dust mite, cat and cockroach) in dust samples. Of these sampled bioaerosols, this project focused mainly on fungi and the findings in relation to fungal concentration levels and their relationship to house and environmental characteristics were

presented elsewhere (Hargreaves et al. 2001). The aim of the work presented in this paper was to examine associations occurring between the concentration levels of submicrometre and supermicrometre particles and fungi concentrations. Existence of associations may on one hand point out to the similarities in the behavior between biological and non-biological particles in the air, and on the other hand could help identifying interactions occurring between these two types of particles. While this would not serve to directly define any relationship between non-viable particles and fungal allergens, it was considered that fungal allergens would be present in the air in direct proportion to the numbers of cultivable fungal spores, particularly those known to have an allergenic effect. Thus, a relationship between particles and allergens could be inferred from an observed relationship between particles and fungal spores.

MATERIALS AND METHODS

House sample selection

The sample of non-air conditioned houses for this study was drawn from a suburb in Brisbane, Australia. Selection of a single, reasonably flat suburb ensured that all houses were subject to similar meteorological conditions. The selected suburb contained a wide cross-section of house building materials and styles: new & old, brick & timber and high-set & low-set. Five hundred invitations to participate in this study were hand delivered to letterboxes. As a result, seventeen householders volunteered, of which fourteen eventually took part. The measurements were conducted during May-July 1999, that is, late autumn and winter in Brisbane.

Experimental design

On arrival of the researchers at the test houses, a two-minute outdoor bioaerosol (fungi and bacteria) measurement was conducted using the Reuter Centrifugal Air Sampler (RCAS) under normal ventilation conditions. Normal ventilation conditions were defined as those that had already been established by the resident prior to the researchers arrival (i.e. all windows and doors that are normally open in the residence for this time of year, were open). The following steps were to collect ten minutes samples of the living room and bedroom bioaerosol first and then to set up one hour continuous and simultaneous measurements in the living room of particle number concentration (using the Condensation Particle Counter (CPC), and Aerodynamic Particle Sizer (APS)) as well as particle mass (using the Dust Trak). In addition, a hand-held vacuum cleaner was used to collect dust samples for approximately ten to fifteen minutes in the bedroom from the lower bedding (mattress), upper bedding (quilt, doona or blanket), and pillow. Dust samples were also collected from the living room and kitchen floors after all particle sampling was completed, to ensure that the act of collection of dust samples did not influence the particle concentration. After one hour, all doors and windows were closed to create minimum ventilation conditions and the CPC, APS and Dust Trak were run for another hour. The total measurement period in each house was five hours, including set up of the instruments, and so it was unfortunately not practicable to allow a long period of time for minimal ventilation conditions to establish. Bioaerosol samples were collected in the living room and bathroom either before or after the particle sampling, to avoid the activity of the RCAS affecting the particle measurements.

The Qtrak (TSI Model 8551) monitor was used to measure the indoor relative humidity and temperature. Outdoor relative humidity and temperature records were also obtained from the Bureau of Meteorology, for the days of sampling.

The CPC, APS and Dust Trak operate at airflows of 0.3 L/minute, 5 L/minute and 1.7L/minute respectively while the airflow for the RCAS was 40 L/min. Since the larger airflow of the RCAS would affect the particle measurements significantly, fungal samples were taken either directly before or after the particle samples. Therefore particle data did not correlate directly in time to the fungi samples.

The outdoor fungal measurements were conducted to establish the characteristics (concentration and speciation) of outdoor fungi in the air outside the houses. Living room and bedroom samples conducted under normal ventilation conditions, served primarily to provide information about the relationship between indoor and outdoor fungal characteristics while those conducted under minimum ventilation were used to determine possible indoor fungal sources. The bathroom samples were included since the damp, frequently humid atmosphere could well serve to encourage the growth of molds. This room was only sampled under “normal” conditions, since these are actually the same as “minimal”, the bathroom being generally much less open to natural ventilation than the remainder of the house. None of the bathrooms tested showed signs of visible mold on any surfaces.

Dust was collected from the bed because it is where people spend approximately six to nine hours a day, coming into close contact with the mattress, quilt and pillow. The living room is another place where people spend their time either watching TV or relaxing. The importance of the kitchen is that it is a food preparation area. Therefore allergen presence in dust in the bed, living room and kitchen is particularly likely to have health implications, which is why these areas in the houses were selected for sampling.

Measurement of Biological Contaminants

a) Fungi and Bacteria

Sampling was performed using the Reuter Centrifugal Air Sampler (RCAS), and the sampling time was two minutes. Tryptone soy agar strips were used to allow for a broad range of viable fungal and bacterial counts. Xerophilic fungi were not measured selectively, due to the lack of availability of a low water activity medium in a form suitable for use in the RCAS. Nevertheless, the RCAS was considered preferable to other available samplers, since it allows the necessary colony growth required to absolutely identify fungal genera. Since Brisbane is a city of humid weather and moderately high rainfall, the proportion of xerophilic fungi was considered likely to be relatively low. The agar strips were incubated at 28^{o}C for three to four days. The total number of colonies, total fungi and total bacteria were counted and the airborne bioburden calculated in terms of colony-forming units per cubic metre air (CFU/m^3).

CFU/m^3 calculated by: $\frac{\text{Number of colonies} \times 25}{\text{Sampling time}}$

The fungal colonies were identified to genus level using sellotape mounts directly from the nutrient agar strips. This method involved pressing sellotape directly onto the fungal colony surface and placing it onto a microscope slide with lactophenol cotton blue dye. Viewed under the microscope, characteristics of hyphae and spores were used in identification. While this method of collection was therefore confined to the cultivable, viable spores alone, it was this portion of the bioaerosol population that was selected for testing, in order to test the relationship between these fungi, and the total particle count, which would also include non-viable biological particles, and inert fungal spores. While such particles as the latter are known to contribute to allergies, they are not able to establish colonies within houses, or infect susceptible individuals. At the time of testing, the ability to form secondary colonies within the

houses was a key interest of the study, since the primary source of airborne indoor fungal spores had not been determined at the time of the study

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Measurement of Non-Biological Contaminants

a) Submicrometre particle range (0.007 – 0.808 μm)

The number concentration of this particle range was measured using the TSI Model 3022A Condensation Particle Counter (CPC). This particle range (0.007 – 0.808 μm) was selected since it covers most of the particles in the submicrometre range. Each of the instrument settings relates to a specific flow rate, chosen for its operation. The data was stored and saved into a file on a laptop computer attached to the CPC.

b) Supermicrometre particle range (0.54 – 19.81 μm)

The number concentration in this particle range was measured using the TSI Model 322A Aerodynamic Particle Sizer (APS). The instrument settings relate to a specific flow rate, chosen for its operation. The data was stored and saved into a file on a laptop computer attached to the APS. Although the range covered by the APS includes a small fraction of the submicrometre range, for the purposes of this paper the material collected by the APS is referred to as the "supermicrometre" particles.

c) Particle Mass Concentration < 2.5μm ($PM_{2.5}$)

The TSI Model 8520 DustTrak aerosol monitor (TSI Incorporated, St. Paul, MN, USA) was used to measure approximation of $PM_{2.5}$ concentrations. This range was chosen as it relates to the US EPA air quality standards. The $PM_{2.5}$ values obtained in this study using the DustTrak, are not actual gravimetric values, as the instrument was not calibrated for the specific aerosol studied, and would need to be re-

calibrated for the ambient indoor and outdoor type aerosol. It was used in this study to provide relative readings.

Statistics

Fungal and non-biological particle concentrations were analyzed using regression analyses. The fourteen houses were divided into groups to perform comparisons between bioaerosol concentrations and the other variables. Independent T-tests were used to analyse differences in bioaerosol concentrations between the above-mentioned groups. When more than two groups were compared Analysis of Variance (ANOVA) was used. Correlation analysis that examines the influence of one variable on the increase or decrease of another variable, was used to analyse the relationship between fungal and other pollutant concentrations. The significance level used in carrying out any statistical testing was 10%. The statistical package used for analysis was SPSS for Windows.

Results

Fungal concentrations and speciation

A summary of the airborne fungal concentrations, consisting of average, minimum and maximum values, and number of houses with airborne fungal concentration greater than or less than the WHO guideline value of 500 CFU/m^3 (World Health Organisation, 1990) at each site is given in Table 1. The high standard deviation values are likely to be due to small sample size. House 14 showed very high fungal concentrations, which exceeded 1000 CFU/m^3 at all sites.

The most frequently isolated fungal genus was *Cladosporium* (over 50%), followed by, less frequently isolated *Curvularia, Alternaria, Fusarium* and *Penicillium*. The percentage of the specific fungi of total, average, minimum and maximum are presented elsewhere (Parappukkaran et al 2001).

The ratio of indoor (normal ventilation) to outdoor fungal concentrations ranged between 0.4 and 0.9 for all houses except house 17, which had an indoor to outdoor ratio of 1.1. The ratio of indoor (minimum ventilation) to outdoor fungal concentrations ranged between 0.2 and 0.8 for all houses, and was higher than 0.5 for only three of the houses.

Statistically significant correlations were observed between the occurrences of different fungal types. In the outdoor sample, *Alternaria* correlated with *Curvularia* (ρ = 0.8, $P < 0.001$) and *Paeciliomyces* (ρ = 0.8, $P < 0.002$). In the living room sample (minimum ventilation), *Cladosporium* correlated with *Alternaria* (ρ = 0.8, $P< 0.001$) and *Fusarium* (ρ = 0.8, $P < 0.001$). *Cladosporium* also correlated with *Alternaria* (ρ = 0.9, $P <0.001$) in the bathroom sample (minimum ventilation).

Non-viable particle concentrations

A summary of the airborne particle concentrations in submicrometre and supermicrometre ranges as well as $PM_{2.5}$ fraction consisting of average, minimum and maximum values, measured in the living room under normal and minimum ventilation conditions is presented in Table 2

Comparison of non-viable particle and fungal concentrations

The submicrometre, supermicrometre and $PM_{2.5}$ concentrations, from all houses are summarized as box plots in Figure 1. The plots show the variability of the particle and fungal concentrations between the

areas sampled and in particular it illustrates that the living room/minimum-ventilation submicrometre, supermicrometre and fungal concentrations are lower than the living room/normal- ventilation concentrations, while concentrations in the outdoor sample are the highest. For $PM_{2.5}$, the living room minimum sample has slightly higher concentrations than the living room normal ventilation sample. The reason for this trend is not fully understood, one of the hypotheses could be the lower rate of removal under minimum ventilation conditions of larger particles that are re-suspended by the activities of the researchers. These particles are not significant in terms of number, but they dominate particle mass of which $PM_{2.5}$ is a measure. The indoor (normal ventilation) to outdoor ratios of the submicrometre, supermicrometre and $PM_{2.5}$ concentrations in all houses were close to one (Morawska et al 2001). This indicates that under normal ventilation conditions the indoor particle concentrations mirror the outdoor concentrations.

Association between particle and fungal concentrations

Due to the experimental design, most particle concentration measurements did not exactly correspond in time to the fungal sample collection; hence, particle data at the closest possible time was used for analysis between fungal and particle concentrations. Regression analysis was performed to examine the relationship between submicrometre particle, supermicrometre particle, $PM_{2.5}$ and fungal concentrations. Since the fungal concentration units are CFU/m^3, for the purpose of regression analysis this was converted into CFU/cm^3. Scatter plots were computed between the total fungal airborne concentration data and each particle concentration data (submicrometre and supermicrometre and $PM_{2.5}$), for the following sites: outdoors, living room normal and minimum ventilation. These plots were used to determine whether a linear relationship existed between the fungi and particle concentrations. The submicrometre and supermicrometre concentrations are expressed in $particles/cm^3$.

Examination of the scatter plots led to a conclusion that there is no linear relationship between these two variables and also that some data points were isolated from the general distribution of the data. Box plots of the total fungal airborne concentration data and each particle concentrations (submicrometre and supermicrometre and $PM_{2.5}$) were computed to check whether these points were outliers. For fungi, it was noticed that house 13 in the outdoor sample and house 14 in the living room normal and minimum ventilation samples were indeed outliers. The airborne fungal concentration for both these houses were above 1000 CFU/m^3 in all sampled areas (except for living room and bathroom minimum ventilation in house 13) with outdoors fungal concentrations exceeding 2000 CFU/m^3 for both houses.

Since no statistically significant relationship between the fungal and particle concentrations were found when the regressions were performed on untransformed data, the data was transformed either by natural logarithm, or square to further investigate potential relationships. The linearity of the scatter plots still did not improve greatly. However, the R^2 and P values improved after the identified outliers were removed.

Weak but statistically significant relationships were found between supermicrometre particles and fungal concentrations, for the outdoors (R^2 = 0.4, P = 0.03) and living room minimum (R^2 = 0.3, P = 0.04) samples. This was obtained after the outliers: house 13 in the outdoor sample and house 13 and 14 in the living room minimum sample were removed from the analysis. House 13 was the only house that had outdoor fungal concentrations of above 3000 CFU/m^3. In the living room minimum sample, houses

13 and 14 had fungal concentrations of above 700 CFU/m^3, while in all other houses the concentration ranged from 163 – 500 CFU/m^3.

For the remaining regression analyses – supermicrometre particles and fungal concentrations in living room normal ventilation sample, submicrometre particles, $PM_{2.5}$ and fungal concentrations in outdoors, living room normal and minimum ventilation samples – no statistically significant relationships were discovered, even when the outliers were removed.

Fungal concentrations as a function of distance from their probable source

A main road to the south and a park to the north border the suburb under investigation. The possible influence of the park on fungal concentrations was investigated. A graph comparing the distance of the houses from the park with fungal concentrations at all sites is shown in Figure 2. The graph shows that with the decreasing distance between the houses and the park, the level of outdoor (and also indoor) airborne fungal concentration increased. In the immediate proximity to the park, fungal concentrations rose up to ~3100 CFU/m^3, whereas for houses more than 150 m away from the park the concentrations of fungi were below 1000 CFU/m^3.

For the purpose of the analyses the houses were divided into two groups, based on their distance from the park: group 1 were more than or equal to 150 m from the park and group 2 were less than 150 m from the park. Independent-sample t-tests were performed (Table 3) to statistically examine the hypothesis that ‘the mean of group 2 is higher than the mean of group 1’. This hypothesis was accepted

in the case of total outdoor fungi and outdoor *Cladosporium* ($P < 0.02$ and 0.02, respectively) and living room total fungi and living room *Cladosporium* concentrations under normal ventilation conditions ($P < 0.04$, and 0.04 respectively) at a 5% level of significance. The hypothesis was also true for outdoor *Penicillium* and *Aspergillus* concentrations ($P < 0.1$ and 0.1 respectively), *Aspergillus* (living room, normal ventilation conditions) ($P < 0.09$), *Cladosporium* (bedroom normal ventilation conditions) ($P < 0.1$), *Alternaria* (living room minimum ventilation conditions) ($P < 0.09$) and total fungi (bathroom minimum ventilation conditions) ($P< 0.07$) concentrations at the 10% significance level.

Discussion and conclusions

The main aim of this study was to examine whether any associations were present between indoor airborne fungal concentrations and indoor non-biological particle concentrations both in the submicrometre and supermicrometre size ranges. While there are different sources responsible for generation of each of these two types of particles, the reasons for the associations could be twofold. On one hand biological and non-biological particles could behave in the air in a similar way and thus respond similarly to the forces acting on, and processes affecting them. It is, for example, expected that biological and non-biological particles of similar sizes would display the same dynamics in the air. On the other hand there could be interactions occurring between the two types of particles, of which the most likely would be coagulation that results from particle collisions. The process is dependent both on particle concentration and particle size and it governed by the rate of diffusion of particles towards each other. The process is for example faster when a small particle of high diffusion coefficients diffuses to a larger particle with a large surface (Willeke and Baron, 1993).

No statistically significant associations between the fungal spore concentrations and submicrometre particle concentrations were discovered under the experimental conditions of this pilot study. While fungal spores are much bigger than non-viable particles in the submicrometre range, it was considered that fungal allergens, which are in the submicrometre range, could be present in the air in direct proportion to the numbers of culturable fungal spores. Thus, a relationship between particles and allergens could be inferred from an observed relationship between particles and fungal spores. Lack of a relationship could mean that either the assumption of a direct relation between the fungal spore and the allergens is not correct or may be attributed to the experimental design.

For the purposes of this study, a direct relationship between the fungal spore and the allergen concentrations was assumed. However, this did not mean that a direct relation between the fungal spore and submicrometre particles, which would include allergen molecules, was expected, since submicrometre particles may arise from a variety of sources other than fungal allergens and debris of microorganisms. In any future studies, direct measurements of the allergens should be conducted for investigations of associations with other non-viable submicrometre particles, particularly those that are not biological in origin.

In terms of the experimental design, the difficulty in measuring fungi and non-biological concentrations at the same time due to the high flow rate of the RCAS, caused a time difference between these two measurements. Both particle and fungi concentrations change with time, and thus any time difference introduced between the two measurements, may affect the outcome when associations are investigated. Additionally, since the rate of change of these two concentrations may not be the same, more reliable

results could be achieved if time series of particle and fungi concentration could be compared. While, however, measurements can easily be conducted to obtain time series of particle concentrations, due to practical and financial limitations, the number of fungal samples that can be conducted for analyses is limited (often to one sample per specific condition). The experimental difficulties discussed are intrinsic problems to fungi/non biological particles investigations. Future experimental designs should target solutions that would enable measurements of these concentrations to take place as close in time as possible, and to attempt to measure time series of both particles and fungi.

A weak but statistically significant relationship was found between airborne fungal concentrations and supermicrometre particle concentrations in the outdoor and living room samples collected under minimum ventilation conditions. This can be attributed to the fact that both fungi and supermicrometre particles occur in the same size range, 5 - 20 μm and it is expected that their dynamics in the air would be similar. While, in fact, fungi are included in the supermicrometre particle measurement, the proportion of viable fungi was so low, compared with the non-viable/non-biological fraction, that it was considered to have negligible influence on the total supermicrometre numbers. No statistically significant relationship was found between $PM_{2.5}$ and fungal concentrations. This is likely to be due to the fact that while $PM_{2.5}$ is a measure of particle mass and thus is biased towards particles of larger sizes that are smaller in number. The similarity between the supermicrometre particles and fungi measurements was that both were measured in terms of number: either number per unit volume of the air (particles) or number of colony forming units (fungi).

An identified similarity in behavior between fungi and submicrometre particles was, that under minimum ventilation conditions both fungal and submicrometre particle concentrations in indoor air are lower than under normal ventilation conditions. In these respects fungi and submicrometre particle behave in a similar manner. However, a difference noted was that while fungal indoor to outdoor ratios were below one, submicrometre particle concentrations had indoor to outdoor ratios closer to one. Therefore, it is possible that different experimental conditions may uncover interactions between fungi and submicrometre particles.

Another similarity in behavior between the submicrometre particle and fungal spore concentrations that was of interest, was the discovery that the fungal spore concentrations were related directly to the distance from the source, in this case, a nearby park in a very similar way in which the submicrometre particles originating from vehicle emissions from a road, were dependent on the distance to the road (Hitchins et al 2000). In both cases, the concentrations were found to decrease almost exponentially with distance from the source. This finding, coupled with the similarities in the box-plot profiles, indicated that the dynamics of the viable and non-viable aerosols are very similar, although not necessarily proving a physical association between the different particles as had been postulated.

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Table 1. Fungal colony forming units per cubic meter of air (CFU/m^3) sampled at 5 sites in homes

	Outdoors	**Living room Normal ventilation**	**Bedroom Normal ventilation**	**Living room Minimum ventilation**	**Bathroom Minimum ventilation**
Average	1133	810	692	453	499
Standard Deviation	759	389	385	389	521
Minimum	463	400	350	163	125
Maximum	3125	1675	1688	1713	2063
No. of homes with <500 CFU/m3	1 (7%) (n = 14)	2 (15%) (n = 13)*	5 (38%) (n = 13)*	12 (86%) (n = 13)*	10 (77%) (n = 13)*
>500 CFU/m3	13 (93%) (n = 14)	12 (92%) (n = 13)*	8 (62%) (n = 13)*	2 (14%) (n = 13)*	3 (23&) (n = 13)*

*Fungi were not sampled at living room and bedroom normal, and bathroom minimum ventilation conditions in house 7. The decision to sample these sites was taken after sampling house 7, which was the initial house sampled.

Table 2. Summary of outdoor and indoor submicrometre and supermicrometre particle number concentrations (particles. m^{-3}), and particle mass concentration ($PM_{2.5}$: µg. m^{-3}), under minimum and normal ventilation condition

	Supermicrometre (× 10^3 particles cm^{-3})			Supermicrometre (particles cm^{-3})			PM2.5 (µg. m^{-3})		
	OD	ID(MV)	ID(NV)	OD	ID(MV)	ID(NV)	OD	ID(MV)	ID(NV)
Average	23.8	12.7	21.7	1.78	1.54	1.74	11.3	12.0	10.8
S.D	14.0	5.56	12.3	1.34	1.16	1.27	4.0	3.9	3.8
Max	52.8	21.3	40.0	3.98	3.74	3.86	18.4	18.0	15.3
Min	5.03	4.85	5.29	0.45	0.46	0.51	4.7	5.4	4.4

Note: OD: Outdoor; ID: Indoor; MV: Minimum Ventilation; NV: Normal Ventilation.

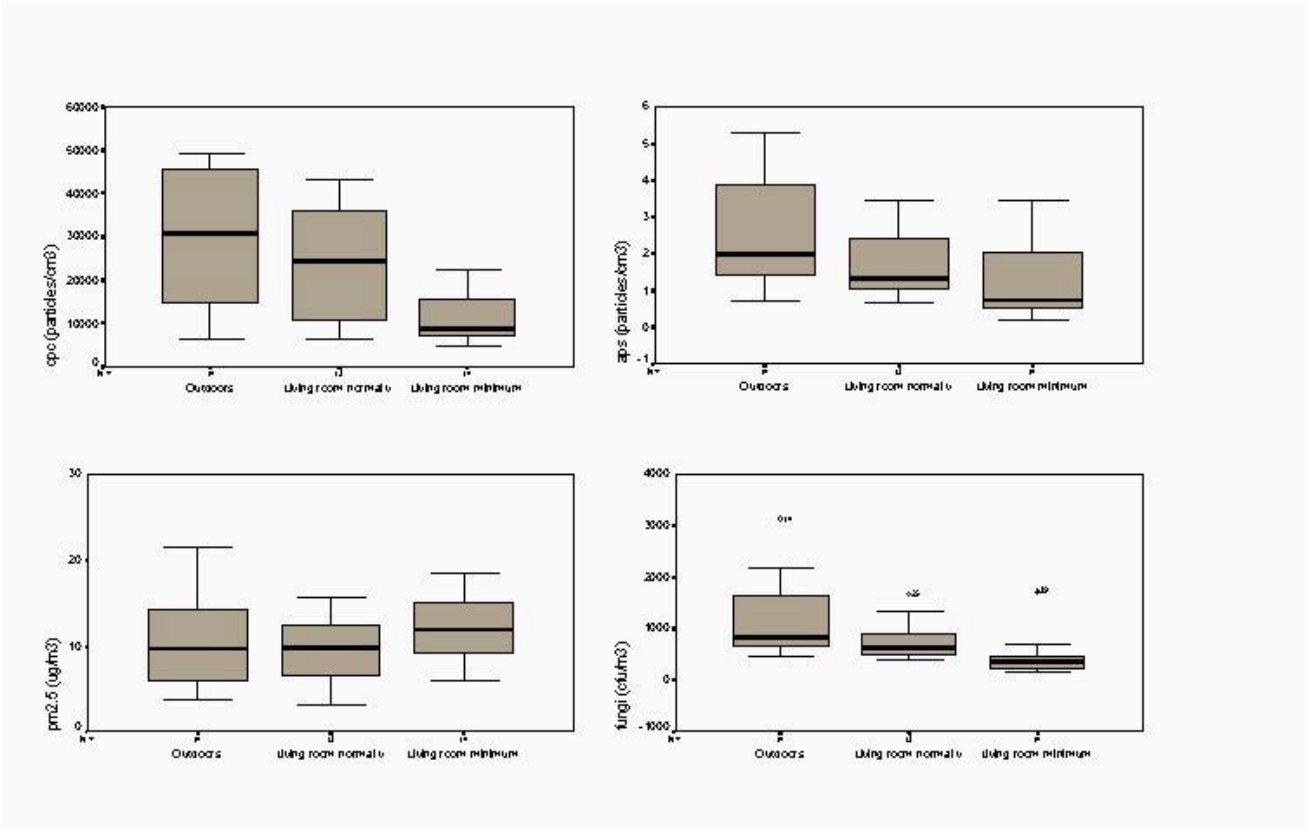
Table 3. Independent sample T-test results for airborne fungal concentration and distance of houses from park. Only those fungi that showed statistically significant relationships with park distance are shown.

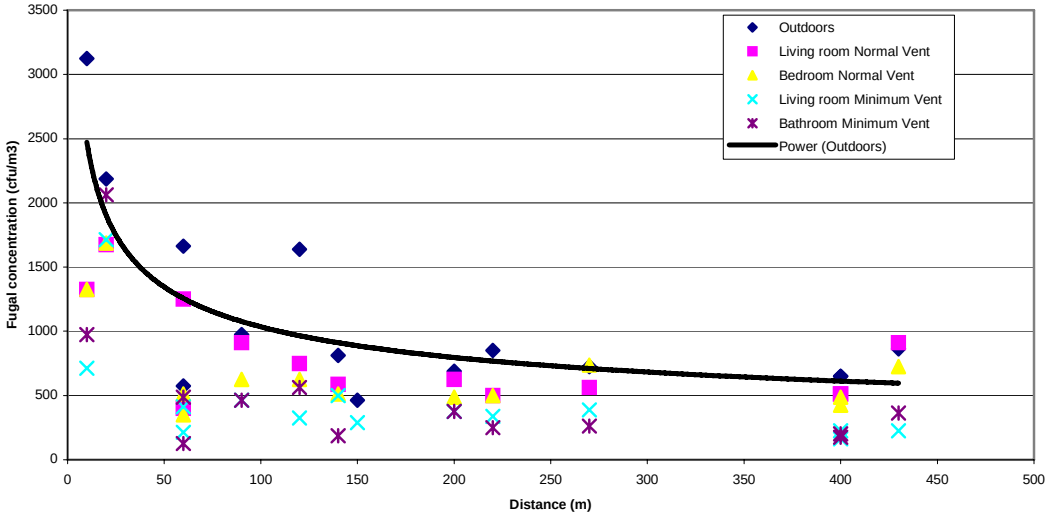
The level of significances (5% or 10%) at which relationships were found are given in brackets next to the *P* value

	Outdoors	Living room normal vent	Bedroom normal vent	Bathroom minimum vent
Total Fungi	*P* = 0.02 (5%)	*P* = 0.04 (5%)		*P* = 0.07 (10%)
Cladosporium	*P* = 0.02 (5%)	*P* = 0.04 (5%)	*P* = 0.09 (10%)	
Alternaria		*P* = 0.09 (10%)		
Penicillium	*P* = 0.1 (10%)			
Aspergillus	*P* = 0.1 (10%)	*P* = 0.09 (10%)		

Figure 1. Box plots showing submicrometre (CPC), supermicrometre (APS), $PM_{2.5}$ and fungal concentrations in outdoor, and living room normal and minimum ventilation conditions.

Figure 2 Fungal airborne concentrations at all 5 areas compared with the distance of houses from the park.


opc (particles/cm3)
aps (particles/cm3)
pm2.5 (ug/m3)
fungi (cfu/m3)
Outdoors
Living room normal v
Living room minimum


Outdoors
Living room Normal Vent
Bedroom Normal Vent
Living room Minimum Vent
Bathroom Minimum Vent
Power (Outdoors)
Fugal concentration (cfu/m3)
Distance (m)
0
500
1000
1500
2000
2500
3000
3500
0
50
100
150
200
250
300
350
400
450
500