



Surface and passive/active air mould sampling: A testing exercise in a North London housing estate



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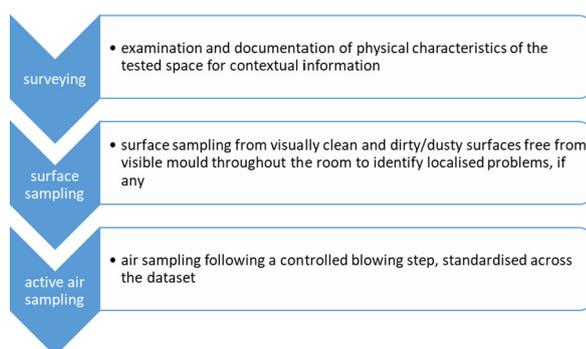
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HIGHLIGHTS

- Mould testing of 71 rooms by both a culture-based and a chemical method.
- A testing protocol that combines air and surface sampling, and particle counting.
- Comparison of passively and actively sampled air readings in relation to the presence of visible mould in the tested rooms.
- Investigation of how physical characteristics of the tested properties relate to the measured mould levels.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite indoor mould being one of the most common problems in residential properties in the UK, there are not any widely accepted methodologies for its measurement. This paper focusses on this problem of measurement and reports on the findings from a rigorous testing scheme carried out to quantify air and surface mould concentrations and particle counts within 71 rooms from 64 properties in North London, some with and some without visible mould. The aim was to investigate the potential of passive and active air sampling strategies (sampling from still and actively mixed air, respectively) to explain visible mould, and understand how home/room characteristics correlate with the obtained readings. Airborne mould levels were quantified using an Andersen sampler (passively and actively), as well as by a chemical method based on the quantification of the *N*-acetylhexosaminidase (NAHA) activity (actively), which was also used to quantify surface mould. The mould levels were then correlated against physical characteristics of the tested homes/rooms, collected by means of survey sheets developed as part of this study. The findings did not reveal any independent variable governing all or most of the response variables, but a complex analysis suggested that whether it is a house or a flat could depict mould levels in the air and on the surfaces. It was also shown that a robust testing protocol should combine air and surface based methods, and an active air sampling strategy leads to a more accurate appraisal of airborne mould levels. Finally, the results showed that while there is some correlation between visible mould (and

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other moisture induced problems such as condensation) and measured air mould concentrations, lack of visible mould within a room does not necessarily mean low air mould concentrations, and thus one should not rely solely on visual inspection.

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1. Introduction

Indoor mould growth is an important issue with critical implications on health and wellbeing, especially in countries such as the UK where the number of homes affected by moisture related problems has been shown to be significant (Sanders, 1989; NIHE, 2011; DCLG, 2015). The problem can be especially serious in areas suffering from growing housing pressures, such as in London. The likely adverse health implications of heavy and systematic exposure to airborne fungal agents, especially in children (e.g. Peat et al., 1998; Mendell et al., 2011), have driven an increase in research in mould and its development in the built environment.

Mould growth is encouraged by a number of complex environmental and biological factors. Experimental research in mould growth in buildings has led to a substantial improvement in the understanding of the specific hygrothermal conditions needed for germination and growth of specific mould species – and these findings have been the basis of the development of substrate-specific isopleths (e.g. Ayerst, 1969; Sedlbauer, 2001) that can be used to evaluate the susceptibility to indoor mould growth of a given indoor environment if no testing data is available (e.g. Abuku et al., 2009; D'Ayala and Aktas, 2016; Aktas et al., 2017). A more in-depth analysis of the presence or concentration of mould within a given indoor environment, however, requires testing using an appropriate strategy and reliable techniques. Testing mainly on mouldy or water damaged buildings is presented by an extensive literature, investigating the effectiveness of cleaning, repair, rehabilitation and renovation works (e.g. Huttunen et al., 2008) or quantifying the impact of ventilation, insulation or indoor finishing materials and flooding/hurricanes on mould contamination (e.g. Riggs et al., 2008; Rabito et al., 2008; Rao et al., 2007). Despite the availability of a wide range of sampling and analysis methods, there are currently no widely accepted, standard protocols to use for measuring mould within a given indoor environment (Aktas et al., 2018). While particulars of each testing protocol can vary, surface sampling is used to detect localised problems on the surfaces, and to gain an understanding of the distribution of mould within an indoor environment. Air sampling however is the most common method used to identify mould presence and, often, its composition (Chew et al., 2006), regardless of the chosen analysis technique. It is considered to be the strongest strategy for mould testing, especially when conducted for health concerns, as it provides a better understanding of airway exposure than surface testing (Méheust et al., 2014). Air sampling can be conducted using both passive (“non-aggressive”) and active (“aggressive”) techniques, i.e. sampling still air, and sampling actively mixed air, respectively¹. Passive sampling can detect the fungal material that is already airborne and is dependent on the level of activity that has taken place within the tested space right before the testing (Swaebly and Christensen, 1952). Active sampling, on the other hand, yields results that are independent of the level of activity, and can also detect mould that is dormant under still conditions, including table-top mould. It was also shown that large

size mould particulates are not detected by means of passive sampling, whereas active sampling makes their presence clear (Maunsell, 1952).

Additionally, while it is widely accepted that certain physical characteristics of indoor environments are somewhat influential on the measured mould levels, the nature of this relationship is far from well-established. Many previous studies have attempted to explore correlations between indoor mould levels and these physical characteristics. While some concluded there are strong correlations between them (e.g. Wang et al., 2016), some argued that home characteristics cannot be used to predict mould presence (e.g. Ren et al., 2001; Verhoeff et al., 1994a, 1994b).

Within this framework, the present study aims to (1) develop insights into what steps a testing protocol should include as a minimum for a thorough appraisal of indoor mould levels, (2) test the capacity of active and passive air sampling methods to explain visible mould, and (3) investigate the impact of certain home/room characteristics on mould concentrations in indoor environments some with and some without visible mould. To these ends, a methodology that combines surface and active/passive air sampling was adopted. Air sampling was undertaken by means Andersen sampler to tally colony forming units (CFU), both actively and passively, and concurrently with this sampling, an additional active air sampling was performed to quantify mould on the basis of on the activity of *N*-acetylhexosaminidase (NAHA), which has been found to be a reliable marker of fungal cell biomass (ASTM, 2014; Reeslev et al., 2003; Reeslev and Miller, 2000; Rylander et al., 2010; Rylander, 2015). NAHA has also been found to be a good indicator of the non-soluble β -glucan, which has certain toxic effects on humans (Nevalainen et al., 2015; Douwes, 2005; Fogelmark et al., 1997; Williams, 1997), and therefore can be used to examine health risks due to fungal exposure (e.g. Terčelj et al., 2011, 2013), **though health implications of indoor mould growth are completely beyond the scope of this study**. Mould measurement based on NAHA activity was quantified by using Mycometer-test, which has been verified by US Environmental Protection Agency (EPA) Environmental Technology Verification Programme (ETV, 2012), and included in Danish Building and Urban Research Institute instructions (Statens Byggeforskningsinstitut, 2003a, 2003b), as well as ASTM 7338-10 (2014). Surface samples were also analysed for NAHA activity. In both air and surface sampling, this study follows the latest Guideline on Assessment and Remediation of Fungi in Indoor Environments released in NY City Department of Health and Mental Hygiene (2000) by the New York City Department of Health as a revision to the 1993 document and then again in NY City Department of Health and Mental Hygiene (2008); these documents advise that indoor remediation work should target not only certain species but all fungi. Based on these guidelines and due to the chemical method selected here, which allows quantification of fungal cell biomass independently from its species (and also because we do not look into the impact of individual species on health), this study does not include species identification, but concerns itself with the total amount of mould present in a given indoor environment.

In addition, particle counts, which were previously found to be potentially correlated to indoor mould concentrations (e.g. Liu et al., 2014; Haas et al., 2013; cf. e.g. Kim et al., 2006), were measured as an additional indicator of the cleanliness of the tested space. These measurements were also done both passively and actively, in order to study its impact on the results. Through these measures, a rigorous testing

¹ While passive and non-aggressive, and active and aggressive terms are often used interchangeably, it has been argued that the terms passive and active might also be used to mean sedimentation and impaction, respectively. It should be noted that this is not the intention here, and in this paper they are used exclusively to denote air sampling from relatively still air and actively mixed air, as explained in subsequent sections.

scheme was carried out in a housing estate in North London, where basic home/room characteristics of the tested properties were also collected by means of survey sheets developed as part of this study.

More details about the testing protocol and tested properties are given in the subsequent sections.

2. Testing protocol

The testing protocol used in this study is described in detail below:

1. **PHYSICAL SURVEY:** This was done via two survey sheets, developed to collect information about the physical characteristics of the tested room and home/building it is located in. The information collected included building age and construction material, whether or not it has insulation, room size and function, and number and size of windows, presence of any visible mould or signs of other moisture problems (condensation, rising damp, leakage etc.), level of furnishing, and level of cleanliness (assigned by the surveyor on the basis of his/her personal judgement). Additionally, descriptive information about the residents was also collected.
 2. **SURFACE SAMPLING:** Surface sampling was performed to gain a more complete view of mould distribution within the tested rooms, and as an additional indicator of the level of cleanliness within the property. In each tested room, 2–5 samples were collected from visually clean surfaces and another 2–5 from visually dusty/dirty, but not mouldy, surfaces. Each area was swabbed with sterile cotton swabs and a 3 × 3 cm adhesive template, and by the same surveyor throughout, in order to maximally standardise the swabbing process. The most common clean surfaces sampled were desks, tables, kitchen counters, or in cases where all horizontal surfaces seemed to be very dusty/dirty, vertical surfaces such as walls and wardrobe doors. The most common dirty surfaces sampled were floors, shelves, and skirting boards. These samples were then tested at the Mycometer laboratories in Denmark to quantify mould by measuring the activity of β -N-acetylhexosaminidase (NAHA) according to a standardised protocol (Mycometer A/S, Denmark). The protocol proceeded as follows: An enzyme substrate containing a fluorophore (4-methyl umbelliferyl) was added to the swab samples. After a reaction time of around 30 min (depending on temperature), the resulting fluorescence formed was measured using hand held fluorometer (Turner Design US /Mycometer version). The fluorescence was measured in relative fluorescence units (RFU) and substrate blank value subtracted. With the chosen calibration of the fluorometer one RFU equals 33.3×10^{-2} pmol 4-MU formed per ml reaction volume per min. The sampling area for the surface samples was 9 cm² and the reaction volume used for analysing surface samples was 2 ml. The evaluation of the surface samples was always performed by the same analyst.
 3. **PASSIVE AND ACTIVE AIR SAMPLING:** Air sampling for NAHA activity was done only in an active way, while Andersen sampling and particle counting was done both in the passive and active ways. Importantly, the passive air testing results reported in this paper were not taken from perfectly still indoor air, as surveyors carried testing equipment in and set these up to do testing, thus creating some minor turbulence in the indoor air. However, the air movement induced by these actions should be the same for all tested rooms. For active sampling, blowing was done by means of a Makita blower with the aim of mixing the air and raising dust in order to mimic some activity level within the room for active air sampling. Blowing duration was graded based on the room size as follows: 1 min for room sizes up to 10 m², 2 min for room sizes up to 20 m², 3 min for room sizes up to 30 m², and 4 min for larger room sizes. The Makita blower was set to speed level 3. First the ceiling, then the walls, and lastly the floor and other horizontal surfaces were blown, keeping the blower approximately 2 m away from the surfaces at all times. The blowing was done by the same surveyor throughout, in order to maximally standardise the process. As the aim was to replicate some level of activity within the room (children running, active cleaning and tidying, or a group of people gathering for an event), and therefore measuring the airborne mould levels heightened by likely daily activity, dust reservoirs that are almost never cleaned or moved around, such as between the lamella of a radiator, were not blown air to avoid generating unrealistic readings. The protocol included a 1 min waiting duration following the blowing, before starting the active air sampling to allow large particles to settle.
- a. **AIR SAMPLING FOR NAHA ACTIVITY:** Active air sampling was made on a MCE-membrane filter, pore size 0.8 μ m using a flow rate of 15 l/min. Sampling was conducted at the centre of the room and for 15 min, giving a sample volume of 225 l. The reaction volume for air samples was 1 ml. The samples were then sent to the Mycometer's labs in Denmark for analysing NAHA activity to determine the mould concentrations. To this end, an enzyme substrate containing a fluorophore (4-methyl umbelliferyl) was added to the filter, and the procedure explained above for surface sampling was repeated. The evaluation of the air samples was always performed by the same analyst.
 - b. **ANDERSEN SAMPLING:** Both passive and active Andersen sampling was done by using a 6-stage sampler with 90 mm Sabouraud 4% glucose chloramphenicol agars. The sampling duration for both passive and active measurements was 5 min. The plates were then incubated at 20 °C for 3 days before colonies were counted to calculate the colony forming units per m³ (CFU/m³).
 - c. **PARTICLE COUNTING:** Particle counting was done using a CEM Particle Counter (Model DT-9880; flow rate 2.83 l/min with 6 channels: 0.3, 0.5, 1.0, 2.5, 5.0 and 10 μ m; counting efficiency equal to 50% at 0.3 μ m and 100% for >0.45 μ m; coincidence loss 5%, 2 m particles/ft³). The device additionally logged temperature (T°) and relative humidity (RH) values at the time of measurement (T° ranging between 0–50 °C \pm 0.5 °C at 10–40 °C and \pm 1.0 °C at other values; RH ranging between 0–100% \pm 3% at 40–60%, \pm 3.5% at 20–40% and 60–80%, and \pm 5% at 0–20% and 80–100%). Particle counting was done both passively and actively for 1 min, and was started simultaneously with the other air samplings.
- The described testing protocol is summarised in Fig. 1. It should be noted that the surveying was done in parallel with sampling tasks, reducing the overall per-room testing duration to ~30 min. Surface sampling is of special note, and was always done before the controlled blowing so as not to disturb the cleanliness levels on the surfaces.

3. Description of tested homes

The testing protocol detailed in Section 2 was used to test rooms in non-water damaged homes in a North London housing estate – these homes were built by and managed under the same administration, and therefore, as explained below in detail, are rather uniform in terms of a number of critical characteristics that are potentially influential on the indoor mould levels, such as building age, materials, construction techniques and detailing, level of maintenance, occupancy, plan typologies etc. This allowed us to be able to concentrate on these homes' divergent properties more in depth.

The estate comprised a total of 64 homes (Fig. 2): studio flats in the North East and South East Blocks (NEB and SEB), 1 and 2 bedroom flats in the North West and South West Blocks (NWB and SWB), and 2 bedroom bungalows. Studio flats were occupied by couples only, while 1–2 bedroom flats and the bungalows by couples with up to two children. The size of the properties varies from 29 m² for studios flats to 105 m² for one of the 1 bedroom flats. Bungalows all measure 53.5 m². All

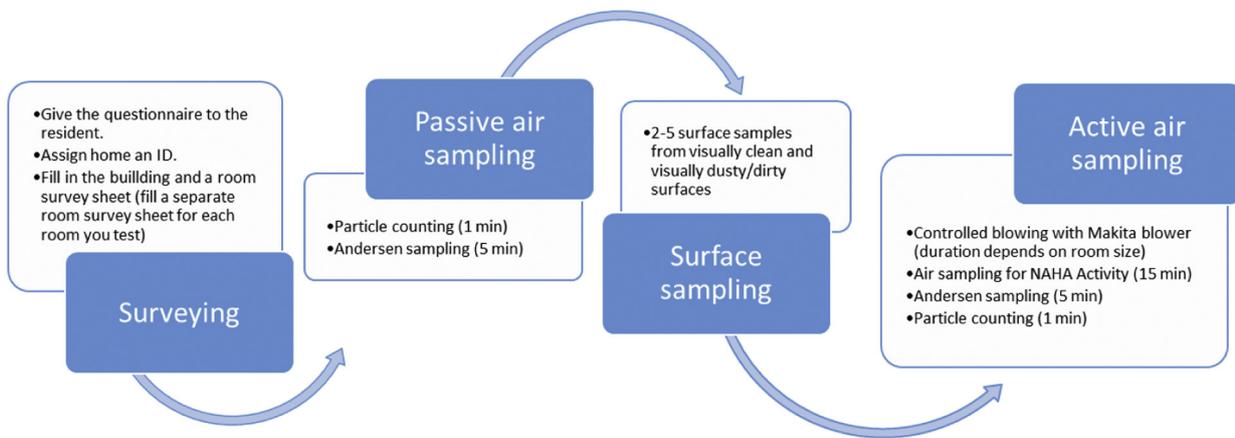


Fig. 1. Test protocol employed for this study.

blocks and bungalows were built in the early 1970's, apart from the NWB and two of the bungalows, which were built in 1992. All are brick masonry cavity wall constructions without insulation. Only the newer NWB was built with cavity wall insulation. In addition, a few bungalows received insulation in 2014. All homes have PVC double glazed windows and central heating systems.

Depending on the property typology and the residents' availability, at least one room was tested in each home visited. As such, the total number of rooms tested in this estate was 71, with 30 studios (multi-functional spaces that act as living room + kitchen + bedroom), 10 bedrooms, 29 combined living room and kitchen spaces, 1 separate kitchen, and 1 separate living room. A summary of the basic characteristics of the tested homes/rooms is in Table 1.

As seen from Table 1, almost 90% of the tested properties date from the 1970's and have no insulation. About half of all homes are either ground floor flats or bungalows. Each category of homes (studio, 1-bed, 2-bed flats, and 2-bed bungalows) have the same plan per category (Fig. 3), with two exceptions: one of these is a detached bungalow to the south of the SEB and SWB, where the tested living room/kitchen area is around 45 m² - the largest room in the dataset. The second irregular case is a property composed of two 1-bedroom flats merged together, so as to create a separate kitchen and a living room, which is unique

in the entire dataset. More than half of the tested rooms have between 20 and 25 m² floor area, and around one fourth is larger. >80% of the tested rooms were used for both sitting and cooking, while more than half of these are also used for sleeping. The percentages of rooms facing four cardinal directions are not very different from each other, while south facing rooms comprise one third of all. Around half of all tested rooms had 4–6 m² of window area. Very few rooms were classified as very dirty (7%) or spotless (6%).

20% of all tested rooms had visible mould inside. These were mostly in the form of small patches, concentrated on and/or around window frames, and black in colour in all cases, and therefore were considered comparable within the dataset. Condensation is the most common of all other moisture related problems, while we did not encounter many rooms with musty smell, paint damage, or leakage stains (Fig. 4). The homes in this housing estate are rented with a standard set of furniture, however families tend to make certain additions to the furniture available within the property – for example a quarter of the properties was found to have rugs or carpets on the floor.

The residents of the tested homes were fully informed about the aims and scope of this study in advance, and asked whether they would like to take part by means of a letter. All of them expressed



Fig. 2. Tested housing estate – top view on Google Earth©. Blocks are shown in blue, while the partly visible buildings to the west of the blocks, surrounding a central garden and shown in yellow, are the bungalows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

A summary of the basic information of the tested properties collected via survey sheets (* If the orientation is NE this was counted both as N and E. ** The reported values here are based on the average of spot T and RH values that were measured simultaneously with passive and active particle counting).

STATISTICS FOR THE SURVEYED HOME AND ROOM CHARACTERISTICS									
<u>Tested home storey</u>		<u>Room function</u>		<u>Room orientation*</u>		<u>Total window area (m²)</u>		1.0-7.7 (4.3±1.6)	
Ground floor (flats&bungalows)	28/64 (43.8%)	Studio (main living + kitchen + bedroom)	30/71 (42.3%)	E	19.8%	<2	10/71 (14.1%)		
1 st floor	19/64 (29.7%)	Living room + kitchen	29/71 (40.9%)	N	25.9%	2-4	19/71 (26.8%)		
2 nd floor	17/64 (26.6%)	Bedroom	10/71 (14.1%)	W	19.8%	4-6	36/71 (50.7%)		
		Kitchen	1/71 (1.4%)	S	34.6%	≥6	6/71 (8.5%)		
		Living room	1/71 (1.4%)						
<u>Construction date</u>		<u>Level of furnishing</u>		<u>Home typology</u>		<u>T at the time of testing (°C)**</u>		22.6±3.0 [range: 17-30.45]	
Built in 1970's	57/64 (89.1%)	With carpet/rugs	18/71 (25.4%)	Studio	30/64 (46.9%)				
Built in 1990's	7/64 (10.9%)	Without carpet/rugs	53/71 (74.7%)	1-bedroom	10/64 (15.6%)				
				2-bedroom	24/64 (37.5%)				
				(flats&bungalows)		<u>RH at the time of testing (%)**</u>		60.4±10.5 [range: 38.2-84.6]	
<u>Room location</u>		<u>Level of cleanliness</u>		<u>Room floor area</u>		<u>Visible mould</u>		14/71 (19.7%) (in 11 rooms (78.6%) in the form of small spots)	
Room in a bungalow	20/71 (28.2%)	Very dirty	5/71 (7.0%)	<10 m ²	7/71 (9.9%)				
Room in a flat	51/71	Dirty	17/71 (23.9%)	10-20 m ²	5/71 (7.0%)				
Southwest Block	12 (16.9%)	Normal	29/71 (40.9%)	20-25 m ²	40/71 (56.3%)				
Northwest Block	7 (9.9%)	Clean	16/71 (22.5%)	25-30 m ²	18/71 (25.4%)				
Northeast Block	18 (25.3%)	Spotless	4/71 (5.6%)	≥30m ²	1/71 (1.4%)				
Southeast Block	14 (19.7%)								
		<u>Room ceiling height</u>		<u>Wall insulation</u>		<u>Other moisture problems</u>		20/71 (28.2%)	
		2.3 m	15/71 (21.1%)	No insulation	54/64 (84.4%)	Condensation		15/20 (70%)	
		2.45 m	37/71 (52.1%)	Insulation	10/64 (15.6%)	Musty smell		3/20 (10%)	
		2.85 m	19/71 (26.8%)			Paint damage		2/20 (10%)	
						Leakage stains		1/20 (5%)	

their consent to the housing estate management for the testing of one or more rooms within their property and their preferred day and time of testing.

4. Data analysis and results

4.1. How visible mould within the tested rooms manifests itself in the air results

The obtained results are summarised in plots shown in Fig. 5.

- Passive readings mostly seem to fail to capture the higher mould concentrations in the air of rooms with visible mould (Fig. 5a). Indeed, our findings show that the difference between passive and active values could be as high as 56 times, which indicates the bias in relying on only passive readings. It should however be noted that although measurements are complete for NAHA based air sampling results and particle counts, there are some missing values for CFU Passive and Active (i.e. 10% of values are missing for the CFU Active and around 50% of values are missing for the CFU Passive). Importantly, the passive Andersen results obtained from non-mouldy rooms vary between 14 and 1138 CFU/m³, which, according to different quantitative recommendations for fungal concentrations released by various organisations, corresponds to a scattered classification from acceptable to contaminated residential indoor environment requiring remediation (Gots et al., 2003).
- While the mean values for air sampling results are a lot higher for rooms with visible mould than those obtained from rooms without visible mould (Fig. 5a&b), the ranges of active CFU and NAHA based air readings obtained from non-mouldy rooms are still quite wide. This means that while presence of visible mould seems to be reflected in the overall high air concentrations, the values obtained from non-mouldy indoor environments are not necessarily low, which might be attributed to hidden mould, which has not been surveyed in this study.

- Fig. 5c shows that the mouldy rooms are overall more particle intensive than the non-mouldy rooms. Further, whether it was measured passively or actively does not seem to be influential on the obtained particle counts, which might indicate that the majority of the particles in the rooms tested here are relatively lightweight, and are suspended in the still air. The fact that passive readings reported here do not come from perfectly still air might have also contributed to this conclusion - the air in the rooms was somewhat disturbed while taking the equipment inside and setting it up. Here too, the ranges are quite wide and overlapping, indicating a high level of uncertainty in the data.

4.2. Relationships between response variables

The seven response variables obtained from the tested properties were analysed using R-Studio (Version 1.0.44 - © 2009–2016 RStudio, Inc.) to examine how these were related to each other: **Air** (actively measured air mould concentrations quantified on the basis of NAHA activity - unit RFU²), **Dirty Surface** (average mould concentrations on visually dirty/dusty surfaces - unit RFU), **Clean Surface** (average mould concentrations on visually clean surfaces - unit RFU), **Particle Active** (active particle count - sum for all 6 channels), **Particle Passive** (passive particle count - sum for all 6 channels), **CFU Active** (number of CFUs from actively sampled air using a 6-stage Andersen sampler - unit CFU/m³) and **CFU Passive** (number of CFUs from passively sampled air using a 6-stage Andersen sampler - unit CFU/m³). Due to the fact that the values of the 7 response variables obtained from the tests are found to vary sustainably and be highly skewed, the natural

² The MMA (Mycometer Air Value) is expressed in relative fluorescence units (RFU) and is a measure of the NAHA (β -N-acetylhexosaminidase) activity obtained when following the Mycometer protocol for sampling and analysis. The NAHA activity is defined as the amount of enzyme which releases 1 μ mol of 4-MU ml⁻¹ min⁻¹. The results are expressed in fluorescence units per m³ air (FLU/m³), where 1 FLU/m³ equals 33.3 \times 10⁻² pmol 4-MU formed per ml per min.

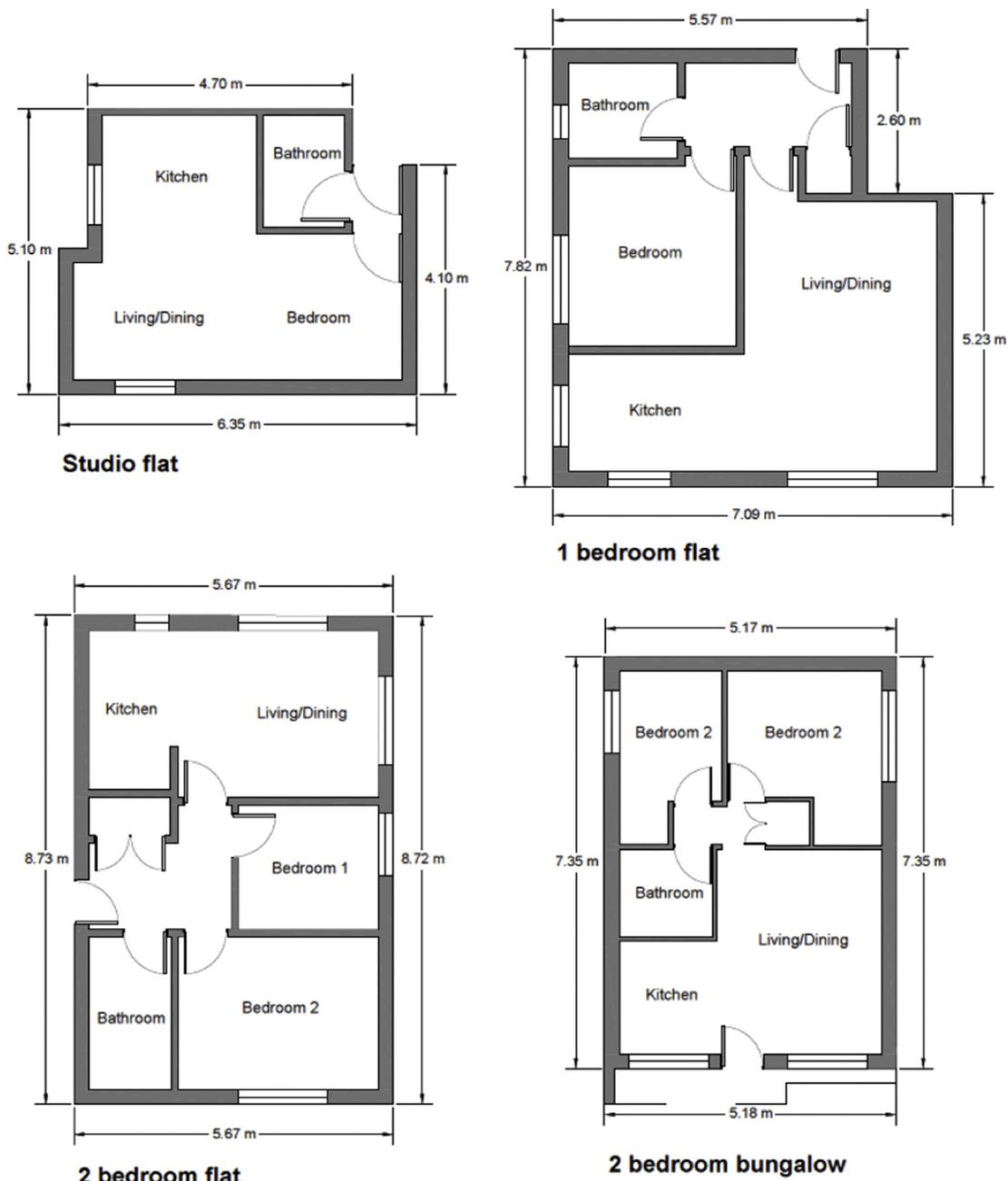


Fig. 3. Plans for different home categories.

logarithm of each variable is used to explore their relationship with each other, instead of the nominal values. This transformation ensures that the skewness is reduced.

It can be seen from Fig. 6 that the highest degree of association is the very strong positive correlation between *Particle_Active* and *Particle_Passive*, followed by the strong correlation between *CFU_Active* and *Air*, and moderate correlation between *Clean_Surface* and *Dirty_Surface*. Further, the *Air* seems to be weakly correlated with the *Clean_Surface* and *Dirty_Surface* and it is almost uncorrelated with *Particle_Active* and *Particle_Passive*.

The correlation between *Particle_Active* and *Particle_Passive* shows that the overall particle intensity in relatively still air and in the actively disturbed air goes hand in hand, as previously discussed in Section 4.1. The correlation between the *Clean_Surface* and *Dirty_Surface*, on the other hand, might be an indication that the table-top dust accumulated on rarely cleaned surfaces is carried around by normal, daily activities

within a given indoor environment, and manifests itself on regularly cleaned surfaces as well.

When the same examination is repeated for the response variables coming only from the rooms where we observed visible mould (Fig. 7), the correlation between *Particle_Active* and *Particle_Passive* becomes stronger, and a moderate correlation appears between the *Air* and *Dirty_Surface*.

CFU_Active and *Air* were found to be correlated ($r = 0.643$) (Fig. 6), to a greater degree for rooms with visible mould ($r = 0.817$) (Fig. 7), despite the different biases inherent in each method. *CFU_Active* values were obtained by using an Andersen sampler, which uses a culture based method with certain drawbacks, including its ability to detect only culturable airborne agents (Maunsell, 1952), therefore potentially underestimating the actual microbial load present in the air (Méheust et al., 2014). It has also been widely argued that culture based test results are highly dependent on incubation conditions and media that



Fig. 4. Some examples of visible mould (top row) and condensation (bottom row) observed in the properties.

might encourage growth of certain species over others (Swaeby et al., 1950). Another problem with culture based methods is that different species grow at different rates and overgrowing may be a problem (Vesper, 2011). Culture based methods have also been shown to be a poor indicator of health impact (WHO, 2009), although this is completely beyond the scope of this study. It should be noted that

NAHA is not specific to fungi and is also produced by bacteria and mammalian cells, making the presence of pollens or pets, as well as the number of inhabitants, potentially influential on the results. However, their impact has been shown to be negligible (Rylander et al., 2010). NAHA is a marker of total fungal cell biomass, able to detect various fungal components. The high correlation between *CFU_Active* and actively

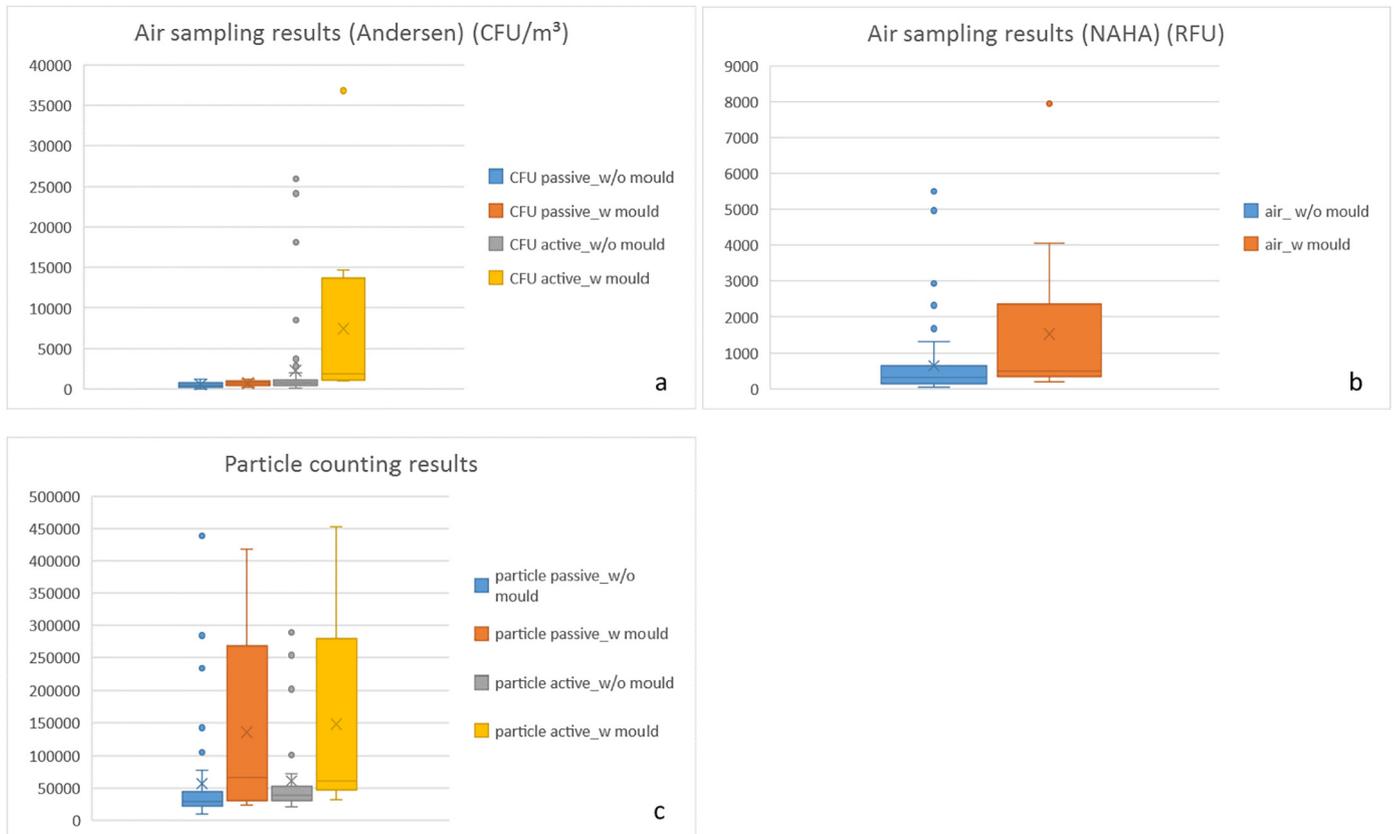


Fig. 5. Summary of obtained readings as per whether there is visible mould or not within the tested spaces.

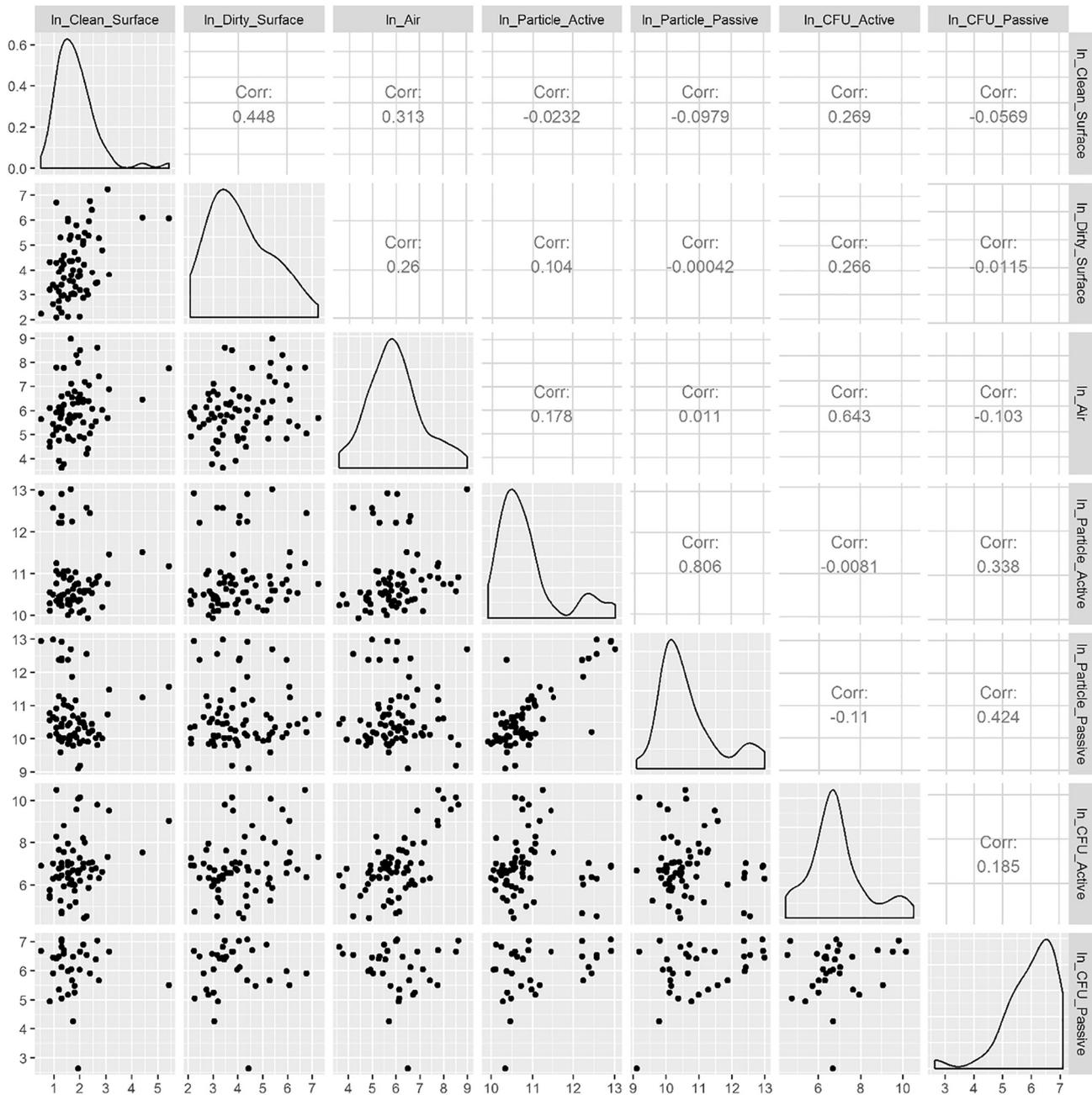


Fig. 6. Distribution of and correlation between the response variables for all 71 rooms.

measured NAHA based Air readings show that, in the dataset under examination, similar proportions of the total bulk of the fungal agent reserve within each tested room were culturable.

The lack of correlation between CFU_Passive and CFU_Active (and hence also Air) highlights once again the unreliability of passive readings, as both were taken using the same culture-based method, i.e. with the same set of drawbacks.

4.3. Relationships between the response and explanatory variables

In this section, the relationships between the previously analysed response variables and the information collected in the physical surveys of the tested properties (explanatory variables) were examined in two stages in order to further investigate how the home/room characteristics affect the response variables. In the first stage a preliminary exploratory analysis aims to identify which characteristics can potentially be important in depicting trends in any of the 7 response variables. Based

on the main conclusions of stage 1, stage 2 adopts a more complex multivariate statistical method, termed Canonical Correlation Analysis, in order to estimate the correlations between multiple response and explanatory variables.

4.3.1. Stage 1: preliminary exploratory analysis

An exploratory analysis was performed by fitting a simple statistical model that relates an individual response variable (i.e., Air, Active_Particle, Passive_Particle, Clean_Surface, Dirty_Surface, CFU_Active or CFU_Passive) with an individual explanatory variable. To this end, 17 explanatory variables were determined from the information collected in the physical surveys of the tested properties as depicted in Table 2. In general, a statistical model has the random component, which expresses the shape of the distribution of the response variable given an explanatory variable, and the systematic component, which expresses the mean response variable as a relationship of the explanatory variable. With regard to the random component, all 7 response

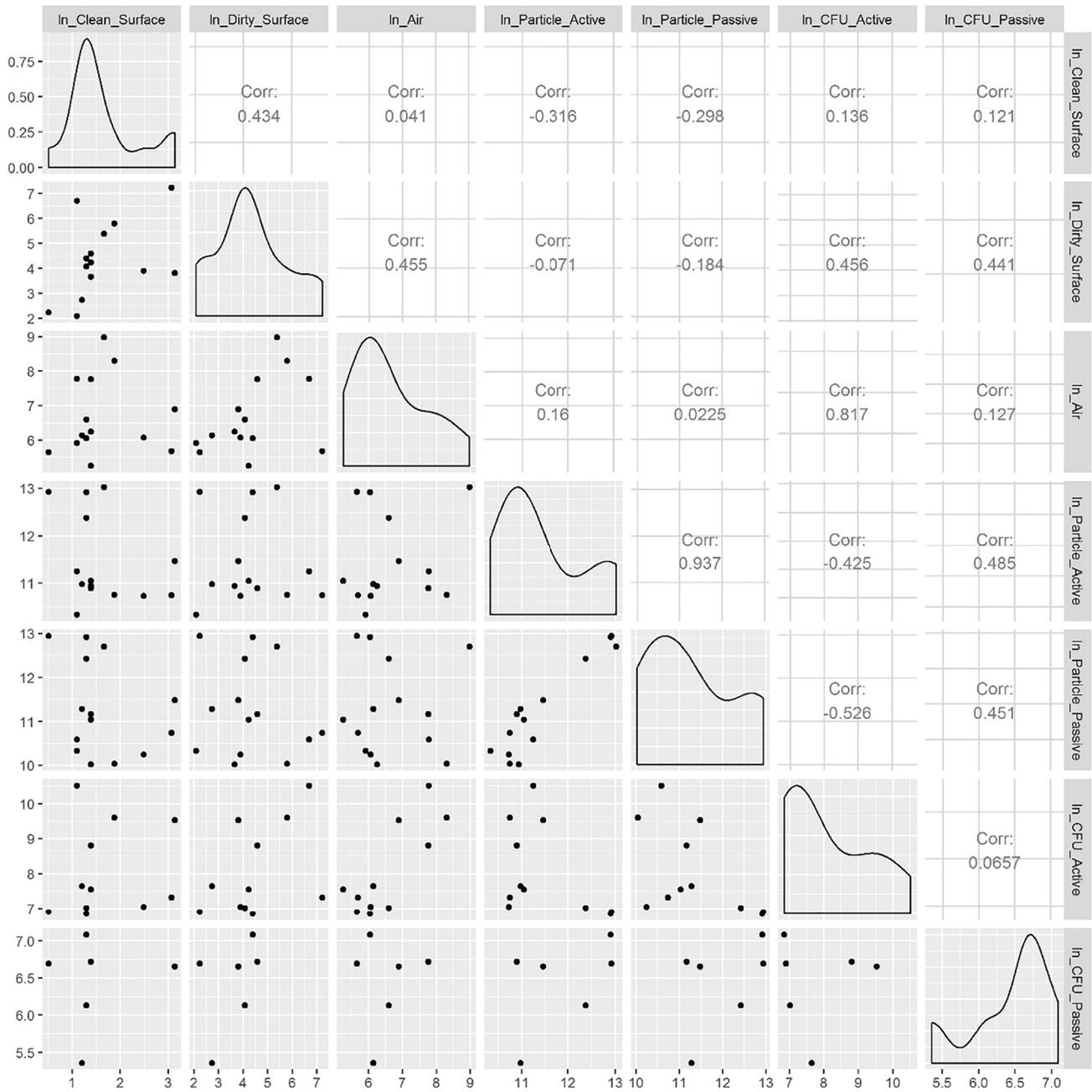


Fig. 7. Distribution of and correlation between the response variables for the rooms with visible mould only.

variables were assumed to follow a lognormal distribution, which is especially evident in the case of Air, CFU_Active, CFU_Passive Clean_Surface, Dirty_Surface (see Fig. 6), and found to fit the data better than its normal, Weibull, and gamma alternatives for all 7 response variables. The systematic component was considered to linearly relate the mean response variable to the explanatory variable. The selected linear model can be written as:

$$\ln(Y) \sim N(aX + b, \sigma^2) \tag{1}$$

where N is the normal probability distribution, a and b and the coefficients of regression, i.e. the slope and intercept, respectively, and σ is the standard deviation; Y is a given response variable and X is a given explanatory variable.

The model was fitted to the data by maximising the likelihood function of the statistical model (i.e., Eq. (1)). A likelihood ratio test was performed next in order to determine whether the selected explanatory

variable is statistically significant. Central to this test is the hypothesis that the examined statistical model fits the data as well as a simpler model, whose systematic component consists of a single intercept. The test produces a p-value. If the p-value is above the 0.05 threshold, then there is not enough evidence to reject the hypothesis and the simpler model is considered to fit the data best. If the p-value is below the 0.05 threshold, the more complex Eq. (1) is considered to fit the data best. In this study, if the p-value is above 0.05, the examined explanatory variable was not considered statistically significant and no further information regarding the analysis was provided in Table 2. By contrast, if Eq. (1) was found to fit the data best, then the estimates of the slope, a, were provided in Table 2 in order to better understand how the change in explanatory variable affects the response variable. The coefficient of determination, R², which shows how well the model fits the data, was also reported.

Table 2 shows that overall no single explanatory variable appears to have a strong or moderate correlation with all response variables. It can

Table 2
Results of the likelihood ratio test ($x = p\text{-value} > 0.05$, $\checkmark = p\text{-value} < 0.05$).

X		Y						
Variables	Categories	ln(Air)	ln(Particle_Passive)	ln(Particle_Active)	ln(Clean_Surface)	ln(Dirty_Surface)	ln(CFU_Active)	ln(CFU_Passive)
Home Type	Flat	x	$\checkmark a: -0.87, R^2: 18\%$	$\checkmark a: -0.70, R^2: 17\%$	x	$\checkmark a: -0.83, R^2: 9\%$	x	x
Home Size	Studio	x	$\checkmark a: -0.42, R^2: 16\%$	$\checkmark a: -0.21, R^2: 8\%$	x	x	x	x
	2 bedrooms		$\checkmark a: 0.39, R^2: 16\%$	$\checkmark a: 0.31, R^2: 8\%$				
Home Floor	2nd Floor	x	$\checkmark a: -0.15, R^2: 9\%$	$\checkmark a: -0.36, R^2: 14\%$	x	x	x	x
	Ground floor		$\checkmark a: 0.46, R^2: 9\%$	$\checkmark a: 0.35, R^2: 14\%$				
Home Orientation	NW and NE Block	x	$\checkmark a: -0.81, R^2: 18\%$	$\checkmark a: -0.61, R^2: 18\%$	x	$\checkmark a: 1.35, R^2: 27\%$	x	x
	SW and SE Block		$\checkmark a: -0.92, R^2: 18\%$	$\checkmark a: -0.79, R^2: 18\%$		$\checkmark a: 0.21, R^2: 27\%$		
Construction Date	1990	x	$\checkmark a: 1.07, R^2: 15\%$	$\checkmark a: 0.97, R^2: 18\%$	x	x	x	x
Insulation	Yes	x	$\checkmark a: 0.80, R^2: 12\%$	$\checkmark a: 0.70, R^2: 12\%$	x	x	x	x
Room Function	Studio	x	$\checkmark a: -0.66, R^2: 14\%$	$\checkmark a: -0.43, R^2: 8\%$	x	$\checkmark a: 0.63, R^2: 6\%$	x	x
Ceiling Height	2.85	x	$\checkmark a: 0.40, R^2: 11\%$	x	x	$\checkmark a: -1.04, R^2: 13\%$	x	x
Carpets/Rugs	Yes	$\checkmark a: -0.66, R^2: 6\%$	x	x	x	$\checkmark a: -0.70, R^2: 6\%$	x	x
Visible Mould	Yes	$\checkmark a: 0.86, R^2: 9\%$	$\checkmark a: 0.79, R^2: 12\%$	$\checkmark a: 0.73, R^2: 15\%$	x	x	$\checkmark a: 1.42, R^2: 15\%$	x
Other Moisture Problems	Yes	$\checkmark a: 0.83, R^2: 11\%$	x	x	x	x	$\checkmark a: 0.79, R^2: 6\%$	x
Temperature		x	$\checkmark a: -0.14, R^2: 21\%$	$\checkmark a: -0.10, R^2: 14\%$	$\checkmark a: 0.07, R^2: 8\%$	x	x	x
Relative Humidity		$\checkmark a: 0.03, R^2: 9\%$	$\checkmark a: 0.04, R^2: 17\%$	$\checkmark a: 0.04, R^2: 25\%$	x	x	x	x
Room Cleanliness		x	x	x	x	x	x	x
Room Orientation		x	x	x	x	x	x	x
Room Window Area		x	x	x	x	x	x	x
Room Size		x	x	x	x	x	x	x

also be noted that the four room properties, i.e., room size, level of cleanliness, orientation and window area, did not depict trends for any of the 7 response variables. Importantly, none of the 17 explanatory variables depicts trends in the *CFU_Passive*. Both *CFU_Active* and *Air* appear to be influenced by the presence of visible mould in the room or the presence of other moisture problems such as condensation, musty smell etc. *Air*, however, appears to be also weakly affected by the presence of carpets or the level of relative humidity in the room at the time of testing. With regard to the average levels of mould obtained from clean and dirty surfaces, it can be noted that despite the relatively strong correlation between these two variables, very different explanatory variables appear to be potentially significant in predicting trends. In particular, trends in the average mould levels on clean surfaces are depicted by temperature at the time of testing. By contrast, the average level of mould on dirty surfaces are influenced by the location of the property and whether it is a flat or a bungalow, as well as the function of the room, the height of its ceiling and the presence of carpets/rugs. Finally, both *Particle_Passive* and *Particle_Active* appear to be influenced by the largest number of explanatory variables, which are the same for both response variables, with the exception of the ceiling height, which is potentially significant only for *Particle_Passive*. In particular, particle counts depend on the home type, size, age and orientation as well as the presence of insulation and function, ceiling height, and the presence of visible mould in the room.

For the explanatory variables found to be statistically significant, their regression coefficients, a , were examined next. In Table 2, a negative a value for flats was estimated by fitting the models, which relate the particle counts and mould on dirty surfaces with the home type (i.e. whether it is a flat or a house). This indicates that rooms located in flats instead of bungalows are expected to have lower particle counts as well as lower average levels of mould measured on dirty surfaces. In addition, rooms used as studios are expected to have on average the lower counts of particles than rooms in 1 or 2 bedroom properties. Rooms in 2 bedroom flats are expected to have on average higher particle counts than rooms in 1 bedroom or studios. Rooms on ground floor properties are expected to have on average higher particle counts than rooms in homes in higher floors. Similarly, rooms on the 2nd floor are expected to have on average the highest particle counts. Particle counts were found to be higher in insulated properties built in 1990.

Rooms covered with carpets or rugs are expected to have on average lower levels of mould in the air and on dirty surfaces. Rooms with visible

mould are expected to have on average higher mould levels of air, active CFU counts, and particle counts. The positive a for the relative humidity indicates that an increase in its level is also expected to lead to higher mould levels in the air and particle counts. Rooms with other moisture problems are expected to have higher mould levels of air and active CFU counts. So far, the explanatory variables had the same positive or negative effect on the different response variables. However, the role of temperature, home orientation, as well as the function and height of the room appears to provide a rather complex picture, as shown in Table 2. In particular, an increase in the temperature in a room is expected to cause a decrease in the counts of particles in the room (i.e., negative a), but an increase in the counts of mould on clean surfaces (i.e. positive a). Rooms with high-ceilings are expected to have higher passive particle counts, but lower mould concentrations on dirty surfaces. Studios are expected to have on average lower particle counts but higher concentration of mould on dirty surfaces than rooms with dual or single uses. Finally, rooms in bungalows are expected to have higher particle counts than rooms in flats. Rooms in flats located in SE and SW blocks appear to have the lowest count of particles. By contrast, studios appear to have the least mould concentration on dirty surfaces and the rooms in the NW and NE blocks appear to have the highest.

Finally, in Table 2 the coefficient of determination (R^2), which shows the proportion of the variation of the response variable explained by the explanatory variable and provides a measure of how well the model fits the data, appears to be low for all regression analyses. This indicates that although some explanatory variables are capable of depicting trends in the response variables, they are able to explain only a small portion of the variation of these variables. This can be attributed, partially at least, to the small sample size of some of the examined variables (e.g. there are only 9 rooms surveyed in the newer properties built in 1990) as well as to the fact that here the importance of individual explanatory variables was explored, and not the partial contribution of multiple variables. This last observation points to the need for a multivariate analysis performed in what follows.

4.3.2. Stage 2: Canonical Correlation Analysis

The Canonical Correlation Analysis (CCA) was used to estimate the correlation between multiple response and explanatory variables (Härdle and Simar, 2007). The aim was to identify whether there is a meaningful and useful relationship between the levels of mould in a room and some room/home characteristics, as well as to

identify which measures of mould and room characteristics contribute most to this relationship. To this end, two synthetic (i.e., response and explanatory) variables were constructed. The synthetic response variable was constructed as the linear function of the natural logarithm of *Air*, *Particle_Active*, *Clean_Surface* and *Dirty_Surface*. It should be noted that the CFU counts were not included in this analysis as they have a notable percentage of missing values, which will further reduce the small sample size. The *Particle_Passive* was also excluded from the synthetic response variable, because it was previously shown that passive and active readings did not vary much (Fig. 5c). The synthetic variable, *Y*, can therefore be written as:

$$Y = a_1 * \ln(Air) + a_2 * \ln(Particle_Active) + a_3 * \ln(Clean_Surface) + a_4 * \ln(Dirty_Surface) \tag{2}$$

where a_i are the standardised canonical function coefficients estimated in order to maximise the canonical correlation. The coefficients are standardised in order to produce a unit variance and be able to be directly comparable. The aforementioned exploratory analysis identified 13 potentially significant explanatory variables. Nonetheless, not all 13 variables can be used in the multivariate analysis due to the relatively small sample size. For this reason, a set of 4 variables that presented meaningful results in the preliminary analyses, as well as the relevant literature, were used to construct the synthetic explanatory variable, *X*, as:

$$X = b_1 * Home_Type + b_2 * Room_Function + b_3 * Visible_Mould + b_4 * Other_Problems \tag{3}$$

where b_i are also the standardised canonical function coefficients associated with the selected explanatory variables. The coefficients are estimated using the package “CCA” (González et al., 2008) using the software R (R Core Team, 2013).

CCA produced 4 canonical functions. In general, the number of produced functions is equal to the smallest number of the observed variables used for each synthetic variable. The first canonical function determines the standardised canonical function coefficients of the linear relationships for which the correlation between the two synthetic variables is maximal. The second function also determines the coefficients, which lead the two synthetic variables to be as strongly correlated as possible given the residual variance left over from the first function and so on.

Overall, the canonical correlation between the two synthetic variables, *Y* and *X*, was found to be high ($R_c = \sqrt{1 - \sum_{i=1}^4 (1 - R_{ci}^2)} = 0.73$). The percentage of variance explained by the full canonical model was measured by the squared canonical correlation coefficient, $R_c^2 = 53\%$, which was also found to be moderate. From these results it can be inferred that there is a meaningful relationship between the levels of mould in the room and the room/home characteristics, chosen to build the synthetic explanatory variable here.

In general, the interpretation of the results is complex and is based only on the statistically significant functions. The *p*-values depicted in Table 3 appear to be well below the 0.05 threshold only for the first function. This indicates that only the first function is statistically significant. Similar to the observations regarding the total model, the moderately high value of R_{ci}^2 ($=0.41$) suggests that there is a meaningful

Table 3
Results for the 4 canonical functions.

Function	R_{ci}	<i>p</i> -Value	R_{ci}^2
1	0.64	~0.00	0.41
2	0.38	0.08	0.14
3	0.28	0.26	0.08
4	0.02	0.89	~0.00

relationship between the selected levels of mould or particulate counts with the house/room characteristics.

Having identified the first function as the most significant canonical one, the study of its standardised canonical coefficients, a_i and b_i , presented in Table 4, follows in order to interpret the results. It should be noted that the categorical variables were transformed into continuous for the needs of the analysis. For simplicity, this was achieved by assigning numerical values: i.e., ‘1’ and ‘2’ to the distinct classes of each categorical variable, as depicted in Table 4, for simplicity. For example, the home type was assigned 1 if it is a flat and 2 if it was a bungalow.

In Table 4, it can be seen that with regard to the observed response variables, the highest coefficient corresponds to *Particle_Active* and it is positive, followed by the also high positive coefficient of *Air*. It can also be noted that the contribution of the *Clean_Surface* and *Dirty_Surface* is moderate and negative. Similar for the observed explanatory variables, the highest contribution to the relationship corresponds to the home type, which is also positive, followed by the also high and positive contribution of the presence of visible mould in the tested room. The presence of other moisture problems appears to contribute little to the relationship and the contribution of the room function appears to be negligible.

Despite the acknowledged complexity in the interpretation of the CCA’s results, for this study, it can be inferred that for rooms in bungalows with visible room and, to a lesser degree, with other moisture problems, are expected to have high measures of mould in the air, as well as high particle counts but lower counts of mould on surfaces. It can also be said that rooms in flats without visible mould or other moisture problems are expected to have lower mould levels on the air and lower particle counts but higher levels of mould on the surfaces. The results indicate that higher air mould concentrations are expected in bungalows and localised mould problems in flats. The results also highlight that, as discussed in Section 4.1, the lack of visible mould does not necessarily mean that the air mould concentrations are low, as mould can be hidden in wall cavities or mould spores accumulated on dirty or clean surfaces. These findings challenge the over-simplistic view that a problem house is the one with visible mould and, in that sense, is in broad agreement with, for instance, Haas et al. (2007), Chew et al. (2003) and Hyvärinen et al. (1993). In light of the above observations, it can be concluded that visual inspection is not adequate to provide an accurate picture of the mould concentration in a room, and testing is necessary.

Overall, the CCA results highlight the overall importance of air sampling as well as the need to integrate air sampling with surface sampling, in order to have a better understanding of the overall mould and particle concentrations and local mould accumulations in any

Table 4
Standardised canonical coefficients for the first canonical function.

Variable	Description	Canonical coefficients
	<i>Y</i>	a_i
<i>ln(Air)</i>	Continuous	0.43
<i>ln(Particle_Active)</i>	Continuous	0.79
<i>ln(Clean_Surface)</i>	Continuous	−0.29
<i>ln(Dirty_Surface)</i>	Continuous	−0.29
	<i>X</i>	b_i
<i>Home Type</i>	1. Flat 2. Bungalow	0.75
<i>Function</i>	1. Bedroom 2. Studio or living room and kitchen	−0.05
<i>Visible Mould</i>	1. No 2. Yes	0.57
<i>Other Problems</i>	1. No 2. Yes	0.16

given room (cf. Duchaine and Mériaux, 2001). In fact, even in the cleanest rooms with no visible mould, surface samples taken from skirting boards showed that these were large reservoirs of mould in very high concentrations – while the average of all mould levels measured on the dirty surfaces was 174.7 RFU, the average from skirting boards was 259.9 RFU (a few surface samples taken directly from visibly mouldy surfaces for comparison purposes showed that the NAHA activity in such surface samples were between 1135 and 2615 RFU). Other surfaces that occasionally resulted in disproportionately high mould concentrations are windowsills and the tops of doors. This clearly shows the importance of integrating surface and air sampling, in order to have an idea of the overall mould concentrations and local mould accumulations, if any.

5. Conclusions

This study reports findings from a testing scheme that combines surface and passive/active air sampling with a culture based and a chemical method to gauge the impact of sampling strategy on the results and seek correlations between measured mould concentrations and various home/room characteristics.

The lack of a strong correlation between results obtained from surface testing (visually clean or dirty) and air testing, as well as the results obtained from canonical analysis show that a testing methodology should not solely rely on surface sampling, while air sampling used alone can hide localised problems. The air and surface based methods should be combined for a robust testing protocol. Further, this study suggests that passive sampling should be replaced by an active testing strategy to fully exploit the predictive capacity of air sampling. The results obtained in this study from passive air sampling were not found correlated to any of the collected explanatory variables, including visible mould or other moisture induced problems.

For the specific dataset analysed here, this study suggests that there is limited correlation between home/room characteristic and the measured mould levels within a given space. There is a moderate association between airborne mould concentrations and visible mould (or other moisture-induced problems). Our findings also show that even the lack of visible mould might not necessarily indicate low levels of airborne fungal agents within a domestic property, and therefore challenges the over-simplistic view that a problem house is the one with visible mould, as once held (see e.g. Richards, 1954). These results demonstrate that one should not rely solely on visual inspection and the only reliable way of understanding the mould levels within an indoor environment is actually testing it.

Abbreviations, symbols and nomenclature

NAHA	β -N-acetylhexosaminidase
RFU	relative fluorescence units
CFU	colony forming units
NEB	North East Block
SEB	South East Block
NWB	North West Block
SWB	South West Block
Air	actively measured air mould concentrations quantified based on NAHA activity
Dirty_Surface	average mould concentrations on visually dirty/dusty surfaces quantified based on NAHA activity
Clean_Surface	average mould concentrations on visually clean surfaces quantified based on NAHA activity
Particle_Active	actively measured particle count
Particle_Passive	passively measured particle count
CFU_Active	number of CFUs per m ³ of actively sampled air using an Andersen sampler

CFU_Passive	number of CFUs per m ³ of passively sampled air using an Andersen sampler
<i>a</i>	Regression slope
<i>b</i>	Regression intercept
σ	standard deviation
<i>Y</i>	response variable
<i>X</i>	explanatory variable
<i>R</i> ²	coefficient of determination
<i>p</i> -value	calculated probability
<i>a</i> _{<i>i</i>}	standardised canonical function coefficients that maximise the canonical correlation
<i>b</i> _{<i>i</i>}	standardised canonical function coefficients associated with the selected explanatory variables
<i>R</i> _{<i>c</i>}	canonical correlation coefficient

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