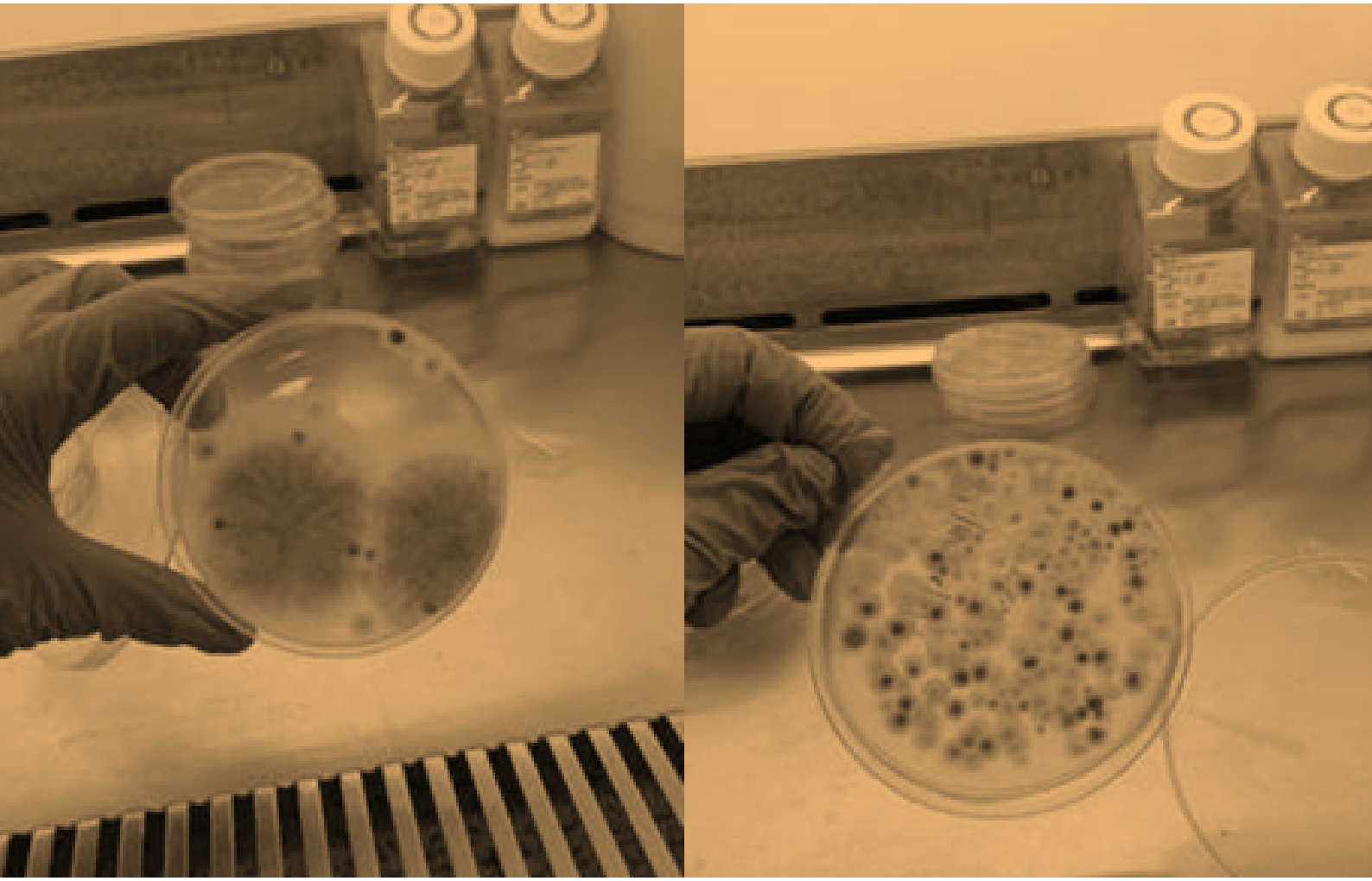
UKCMB

UK CENTRE FOR MOISTURE IN BUILDINGS

INDOOR MOULD TESTING AND BENCHMARKING:

A PUBLIC REPORT



By Yasemin D. Aktas, Hector Altamirano, Ioanna Ioannou, Neil May, Dina D'Ayala

February 2018

ACKNOWLEDGEMENTS

The work reported here was carried out as part of “Indoor Mould Testing and Benchmarking” project, developed at the UK Centre for Moisture in Buildings (UKCMB). This project has been jointly funded by Polygon UK and an EPSRC Impact Acceleration Award (grant reference: EP/ K503745/1). The authors are grateful to both funding bodies for making this collaboration possible. We thank Polygon UK’s Jeremy Skyes and Mike Waterfield, who played a crucial role in the development of the project content. The indispensable in-kind contribution of Mycometer’s Morten Reeslev with testing equipment and sample analyses is kindly acknowledged. Some of the instruments used in this study were borrowed from the UCL CEGE’s Healthy Infrastructure Research Group; we would like to thank Dr Lena Ciric and Dr Melisa Canales for their kind help and advice throughout this work. We are indebted UCL’s Prof Richard Chandler for his advises on the statistical methods utilised in the data analysis. We thank UCL’s Madalina Hanc, and Polygon UK technicians Mike Purser, Robert Plater and Joshua Parris for their help in testing, and Dr Victoria Stephenson for her help in the digitisation of the collected data.

Finally, we send our most cordial thanks to the residents of the tested properties, who were extremely accommodating and helpful.

EXECUTIVE SUMMARY

The understanding of indoor mould is complex and sometimes contentious. Reputable organisations including the World Health Organisation (WHO), the US based Institute of Medicine (IoM) and the National Health Service (NHS) all affirm that high levels of mould in buildings are unhealthy for occupants, and can lead to respiratory and other health problems. Furthermore, moulds can clearly cause fabric decay as well as surface degradation and staining. The environmental conditions and building context within which such moulds develop and affect the building fabric are relatively well understood. However, moulds which might affect human health are not so well understood, partly because the connections between moulds and health are highly complex, but also because we do not currently have a standardised or robust method of measuring indoor mould, or benchmarks for what is a "normal" or "acceptable" level in houses. The research outlined in this report is an attempt to address this problem of measurement, and provide a way forward for research in this area.

Why is it difficult to measure mould levels in buildings? The main reason is that most mould is not visible, but is airborne. So in order to understand the condition of a house, we have to measure the levels and perhaps also the types of mould in the indoor air (as well as sampling any visible mould). But how do we measure mould in the air? And what type or part of mould should we be seeking to measure? How do these also relate to mould on surfaces? It is because these questions have not been adequately answered in independent rigorous research that it is currently impossible to say what an acceptable level of mould in a building is, and at what point mould concentrations exceed this level.

This project involved an extensive literature search of mould testing techniques and methodologies, a programme to test certain methodologies, an analysis of the effect of environmental and lifestyle factors on the tested mould levels, and finally an attempt to benchmark mould levels under the chosen test protocol. The conclusions to the project are as follows:

1. There are currently no established techniques for mould testing which would allow proper comparison of test results reported by different studies, and enable the benchmarking of mould levels in buildings. The establishment of an accepted methodology and protocol for testing mould levels is essential if meaningful research into the effects of mould on human health, and the relationship between building condition and mould levels, is to be possible.
2. It is unclear which mould species or parts of moulds (such as spores, cell fragments, mycotoxins, beta glucans) affect human health and therefore should be measured. It is estimated that there are over a million species of mould, and indoor air will normally contain thousands of mould spores in each cubic metre, but there are no clear definitions or methods of testing for most of them. Furthermore it is unclear how the human body responds to different types and parts or combinations of moulds, as this is also dependent not only on exposure but on other conditions of the individuals concerned (such as age, health, genetic make-up, lifestyle, occupation). In the context of this considerable uncertainty and complexity, the method that we used in this pilot research for assessing mould in buildings (following guidance from New York City Department of Health, among others) is to measure the general level of all types and parts of moulds, rather than individual species or parts[1].
3. This general level testing was achieved by a measurement technique based on the quantification of the activity of N-acetylhexosaminidase (NAHA), an enzyme which has been found to be a reliable marker of fungal cell biomass. This testing was carried out using an active (aggressive) sampling method. We also field-tested passive (non-aggressive) and active (aggressive) sampling techniques using a culture-based and a particle counting method. On the basis of these results as well as an analysis of the literature, it appears that passive testing of the air in buildings is unable to capture visible mould in the

[1] At the same time, but within a different project, the UKCMB have been conducting an extensive analysis of the relationship between Health and Moisture in Buildings, which covered not only damp but also over-dry building conditions, and not only moulds but also bacteria, VOCs and other agents of disease. For more information: http://www.ukcmb.org/health-and-moisture-in-buildings-report

tested room, and can greatly underestimate the concentration of moulds within a given indoor environment. It is one of the conclusions of this project that active sampling methods should therefore be used in mould testing. Active methods involve controlled air-blowing in order to disturb mould fragments in the room being tested and thereby provide greater consistency and reproducibility to air sampling, while at the same time replicating more realistically some level of human activity in a room, and making obtained readings more representative of the actual exposure levels.

4. The combined methodology of active air sampling and particle counting as well as surface sampling from visually clean and dirty/dusty surfaces, together appear to be a reliable basis for mould testing in a room. The air and surface readings obtained from indoor environments with no visible mould at all were then analysed using a benchmarking methodology used by the Danish Building Institute. Importantly, benchmark values suggested here are only valid using the exact same testing protocol, and were found quite similar to benchmarks previously established (and currently in use) in Denmark by using the same methods of testing and analysis.
5. However, other active testing methods and types of sampling may also allow benchmarking. From a research perspective, it is important that the majority of research work strictly follows the same protocols, whatever these are, so that results are comparable and a significant body of data can be built up.
6. The results show a clear difference between mould concentrations obtained from homes with visible mould and those without. These benchmarks could be used to identify the degree of the mould infestation, and, possibly, with further research, hidden (i.e. non-visible) mould infestation.
7. Through survey sheets and questionnaires, developed here and used in conjunction with the testing methodology, this research also looked into how building condition, context (e.g. age, location, construction type, insulation, function and level of furnishing) were related to mould levels, particularly where mould levels are high. This could lead to positive measures to reduce mould levels through changes to the building and behaviour. However, in this short study we found only very limited correlations, which indicates the need for a greater and longer testing programme.

It should be stated again that this project is very much a first step in our attempts to test and benchmark mould levels in buildings, and, by doing so, to bring greater clarity to the relationship between buildings, mould and people. It is important that we build on this first project with further research both to widen and to challenge these early findings, so that moisture safety in buildings can be taken forward in a logical and proven manner.

Neil May, October 2017

UKCMB

TABLE OF CONTENTS

This report summarises the activities and findings of the pilot project "Indoor Mould Testing and Benchmarking", which was conducted between May 2016 and May 2017, and identifies the areas that require attention by further research. The project was facilitated by the UK Centre for Moisture in Buildings (UKCMB), funded by Polygon UK and the EPSRC Impact Acceleration Funds (grant reference: EP/K503745/1) and implemented by UCL Civil, Environmental Geomatic Engineering (CEGE) with support from UCL Institute for Environmental Design and Engineering (IEDE) and Mycometer.

1. AIMS AND OBJECTIVES

Indoor mould growth is an important issue with implications for health, economics, and wellbeing. In countries such as the UK, there is now considerable concern about mould in homes, as shown by the recent All Party Parliamentary Group for Healthy Homes and Buildings Report[2] which states that "around a third of people living in the UK report that they have mould in their homes".[3, 4] Mould is encouraged by sustained high levels of humidity within and around building envelopes, causing degradation of the building materials, poor air quality, and problems with respiratory health[5]. Thus, there is a growing amount of research carried out in the field, driven mainly by possible adverse health implications of heavy and systematic exposure to airborne fungal agents, especially in children.

Despite being an extremely common phenomenon in properties throughout the UK it is still not clear how mould growth should be measured, and what background levels of mould may be considered normal & acceptable. This research focusses on this problem of measurement.

Within this framework, the aims and objectives of this study are given below:

i. Assessment of existing mould testing techniques and methodologies, and a synthesis of the literature for reasons that might be inhibiting the formation of a well-grounded methodological basis;
ii. Testing out various sampling methods and comparing them;
iii. Exploring the impact of various physical and lifestyle related characteristics on the measured mould levels within a given indoor environment;
iv. Benchmarking the "normal" or "typical" mould levels for the UK buildings with a proposed methodology.

2. LITERATURE REVIEW

In order to have an understanding of mould testing methods and analysis techniques, and also for an accurate appraisal of problems associated with mould research from a built environment perspective, a thorough analysis of the existing literature on the mould research was carried out. To this end, more than 250 publications including peer reviewed academic journals and books were examined, those that focus explicitly on epidemiology were left out, and a synthesis of the remaining +90 resources was made and presented in the form of a report.

This report shows that the vast majority of the extant research discourse is from and intertwined with a health perspective, leaving the body of research focussing on the built environment particularly wanting. Studies focussing on the impact of current energy retrofitting or insulation practices on the susceptibility of a given indoor environment to grow mould or on the constructive features encouraging mould growth are extremely scarce, if present at all.

[2] APPG (2017). Building our Future Laying the Foundations for Healthy Homes and Buildings.
[3] Energy Saving Trust (2014). Cold, draughty, mouldy, damp: What the UK public think about their homes.
[4] This figure should however be balanced by the English Housing Survey estimate of only 4% of homes affected by mould, a significant decline from 13% of homes in 1996.
[5] See, for example: World Health Organization (2009). WHO guidelines for indoor air quality: dampness and mould. Copenhagen: WHO; 228p, and Institute of Medicine (2004). Damp indoor spaces and health. Washington, DC: National Academies Press; 355 p

The literature review also included a critical analysis of the main methods of analysis for an in-depth appraisal of strengths and biases of each (Table 1[6]).

Table 1: A summary of main mould analysis methods with their respective advantages, disadvantages and notes in relation to their feasibility, accessibility and costs

Methods of analysis	Notes	Advantages	Disadvantages / Limitations
Polymerase chain reaction (PCR) based methods	PCR amplification and DNA sequencing. Both for surface and air testing. Non-viable fungi can also be determined. Targeting directly DNA in fungal particles. Mold-Specific Quantitative PCR (MSQPCR) has been developed for +100 species[7] and is used to calculate Environmental Relative Moldiness Index (ERMI)[8].	Low detection limit and high accuracy[9]. Very sensitive[10]. It has future promise in terms of specie detection which may be crucial especially within the context of health implications.	Sample contamination[9]. Lack of high-quality reference sequence for fungi apart from an estimated 1% of all species[11]. Sampled fragments might be lacking DNA and it does not indicate whether the collected mould is viable[10]. MSQPCR assays are species specific[7].
Microscopic methods	Helps identify whether collected mould is fresh, desiccated or dead. Both for surface and air testing by means of slit or impactors.	Direct, quick and able to quantify both dead and living microorganisms. It helps differentiate between colonisation (fruiting structure or hyphal penetration) or tabletop mould (mixed spores)[10].	Difficult to differentiate between some common genera, also requires trained staff, labour intensive and costly[9]. It does not indicate whether the collected mould is viable[10].
Culture based methods	Andersen samplers are well established and common for air sampling. Among commonly used samplers are other impactors, liquid impingers and filters.	Economical and able to differentiate between common genera[9]. It allows the identification of the species[10].	Obtained results are highly-dependent on incubation conditions and medium, which might encourage growth of certain species over the others[12,13]. Different species grow at different rates[7]. Applicable to only viable and culturable fungi[14] Time-consuming[10]. Over-growing is a potential problem[7].

[6] Full literature review report can be obtained from UKCMB.

[7] Vesper, S. (2011). Traditional mould analysis compared to a DNA-based method of mould analysis. Critical Reviews in Microbiology, 37(1), pp. 15-24.

[8] Vesper, S. et al. (2007). Development of an Environmental Relative Moldiness Index for US Homes. Journal of Occupational and Environmental Medicine, 49(8), pp. 829-833.

[9] Méheust, D. et al. (2014). Indoor fungal contamination: health risks and measurement methods in hospitals, homes and workplaces. *Critical Reviews in Microbiology,* 40(3), pp. 248-260.

[10] Horner, W. E., Barnes, C., Codina, R. & Levetin, E. (2008). Guide for interpreting reports from inspections/investigations of indoor mold. Journal of Allergy and Clinical Immunology, 121(3), pp. 592-597.

[11] Begerow, D., Nilsson, H., Unterseher, M. & Maier, W. (2010). Current state and perspectives of fungal DNA barcoding and rapid identification procedures. Applied Microbiology and Biotechnology, 87(1), pp. 99-108.

[12] Swaebly, M. A., Christensen, C. M. & Grahek, T. A., 1950. Tests of different media for the collection and identification of air-borne saprophytic fungi. *Journal of Allergy,* 21(5), pp. 404-408.

[13] Ren, P. et al., 2001. The relation between fungal propagules in indoor air and home characteristics. *Allergy,* Volume 56, pp. 419-424.

[14] Maunsell, K., 1952. Air-borne fungal spores before and after raising dust (sampling with sedimentation. *International Archives of Allergy,* Volume 3, pp. 93-102.

Chemical and immunoassay methods	a. Methods targeting cell wall components: glucans and ergosterol measurements. b. Methods targeting Microbial Volatile Organic Compounds (MVOCs). c. Chemical methods can target certain specific components, such as NAHA (β-N-acetylhexosaminidase enzyme) which has been shown to be a marker of fungal biomass[15,16]. Applicable to both air and surface testing.[17]	a. Glucans, a fungal cell component, are known to have toxic and immune-suppressing effects, therefore are a meaningful fungal component to target in studies focusing on health implications[18,19,20,21]. Ergosterol is a good marker for mould[22]. b. MVOCs may be responsible of nonspecific symptoms. c. NAHA presence has been found correlated to the non-soluble fraction of β-glucan[23], and therefore can be used to assess toxicity. Certain health problems such as sarcoidosis and nocturnal asthma were found correlated to high levels of NAHA exposure[24,25].	a. Most, but not all, species that are common indoors are significant source of glucans[26] which are not specific to moulds, but can be present in other plant cells[9], therefore testing methods targeting glucans may overestimate exposure. Ergosterol measurements do not provide information on species[8]. Testing is difficult and costly, and not performed in most laboratories[22]. b. Low amounts of MVOCs emitted by mould. Specie-nonspecific. c. NAHA is not specific to fungi[23] and can be produced by bacteria, fungi, protozoa[24], and even mammalian cells therefore is influenced by presence of pollens or pets as well as number of inhabitants[27]. Furthermore, it is produced more by filamentous fungi, e.g. Aspergillus genera[26].

[15] Madsen, A. M., 2003. NAGase activity in airborne biomass dust and relationship between NAGase concentration and fungal spores. *Aerobiologia,* Volume 19, pp. 97-105.
[16] Reeslev, M., Miller, M. & Nielsen, K. F., 2003. Quantifying Mold Biomass on Gypsum Board: Comparison of Ergosterol and Beta-N-Acetylhexosaminidase as Mold Biomass Parameters. *Applied and Environmental Microbiology,* 69(7), pp. 3996-3998.
[17] Apart from these, there are also Methods targeting mycotoxins: gas spectrometry, enzyme-linked immunoabsorbent assay (ELISA) and allergens.
[18] Nevalainen, A., Täubel, M. & Hyvärinen, A., 2015. Indoor fungi: companions and contaminants. *Indoor Air,* Volume 25, pp. 125-156.
[19] Douwes, J., 2005. (1→3)-β-D-glucans and respiratory health: a review of the scientific evidence. *Indoor Air,* 15(3), pp. 160-169.
[20] Fogelmark, B., Sjöstrand, M., Williams, D. & Rylander, R., 1997. Inhalation toxicity of (1→3)-β-D glucan: recent advances. *Mediators of Inflammation,* 6(4), pp. 263-265.
[21] Williams, D. L., 1997. Overview of (1→3)-β-D-glucan immunobiology. *Mediators of Inflammation,* Volume 6, pp. 247-250.
[22] Portnoy, J. M., Barnes, C. S. & Kennedy, K., 2004. Sampling for indoor fungi. *Journal of Allergy and Clinical Immunology,* 113(2), pp. 189-198.
[23] Rylander, R., 2015. β-N-Acetylhexosaminidase (NAHA) as a Marker of Fungal Cell Biomass – Storage Stability and Relation to β-Glucan. *International Journal of Environmental Monitoring and Analysis,* 3(4), pp. 205-209.
[24] Terčelj, M., Salobir, B., Harlander, M. & Rylander, R., 2011. Fungal exposure in homes of patients with sarcoidosis - an environmental exposure study. *Environmental Health,* 10(8), pp. 1-5.
[25] Terčelj, M. et al., 2013. Nocturnal asthma and domestic exposure to fungi. *Indoor and Built Environment,* Volume 22, pp. 876-880.
[26] Iossifova, Y. et al., 2008. Use of (1-3)-β-D-glucan concentrations in dust as a surrogatemethod for estimating specific fungal exposures. *Indoor Air,* Volume 18, pp. 225-232.
[27] Rylander, R., Reeslev, M. & Hulander, T., 2010. Airborne enzyme measurements to detect indoor mould exposure. *Journal of Environmental Monitoring,* Volume 12, pp. 2161-2164.

The key findings from the literature review are summarised below:

i. There is not a single method that is found sufficient for all applications and all types of mould growth; each has strengths, biases and weaknesses in different contexts;

ii. There are several methodologies currently in circulation, but, despite their credentials, these have so far not attracted a widespread acceptance from interested parties/stakeholders;

iii. Three systemic problems in the literature are (1) a wide range of different testing protocols used in the literature, which do not produce comparable results; (2) the almost universal use of passive air sampling[28], which provides inconsistent results depending on the level of activity that has just taken place in the tested room prior to testing, or which, in case no activity is allowed within the room to be tested prior to the measurement, underestimates the actual mould reserve; (3) interpretation based on a comparison of few indoor-outdoor measurements, which, due to the lack of a well-grounded understanding of background mould levels, has been shown also to be highly random.

3. TESTING PROGRAMME

In order to achieve the aims, a rigorous testing programme was designed and implemented. More information on the methodology and the testing protocol, as well as the description of the tested homes, are given in the subsequent sections.

3.1. Methodology

A methodology that was previously developed and used by the Danish company Mycometer was adopted for the purpose of this research. Mycometer's original methodology combines surface and air sampling, using an active (aggressive) air sampling strategy. This methodology has been extended as part of this pilot project by integrating a thorough survey of the tested property and passive and active particle counts to measure indoor particle intensity. This allowed for a protocol (Figure 1) able to detect localised problems and produce airborne fungal agent concentration values that were shown to capture the presence of visible mould and other indicators of moisture damage within the tested rooms. The mould levels on surfaces and in the air were quantified by using activity of the enzyme β-N-acetylhexosaminidase (NAHA)[29], which has been shown to be a biochemical marker for mould biomass.

[28] While terms "passive" and "non-aggressive", and "active" and "aggressive" 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 report they are indeed used to point out air sampling from relatively still air and actively mixed air, as explained in subsequent sections.

[29] Mycometer's analysis method is based on the quantification of the activity of an enzyme, β-N-acetylhexosaminidase (NAHA), according to a standardized protocol (Mycometer A/S, Denmark). This analysis method is approved by the Danish Building Research Institute, and by ASTM D7338-14. A number of peer reviewed academic papers have been written on this methodology and its results. NAHA activity has been used the last 20 years, in research and by the mould remediation industry to estimate the level of mould on surfaces and in air. This method was selected because of the need for a knowledgeable partner in this project. However, as with all the methods examined it has both advantages and disadvantages, which is why we also selected other methods and why we are not claiming that this method is the only one that should be used in the future. An assessment of different methods was made as part of the literature review, and is available on request.

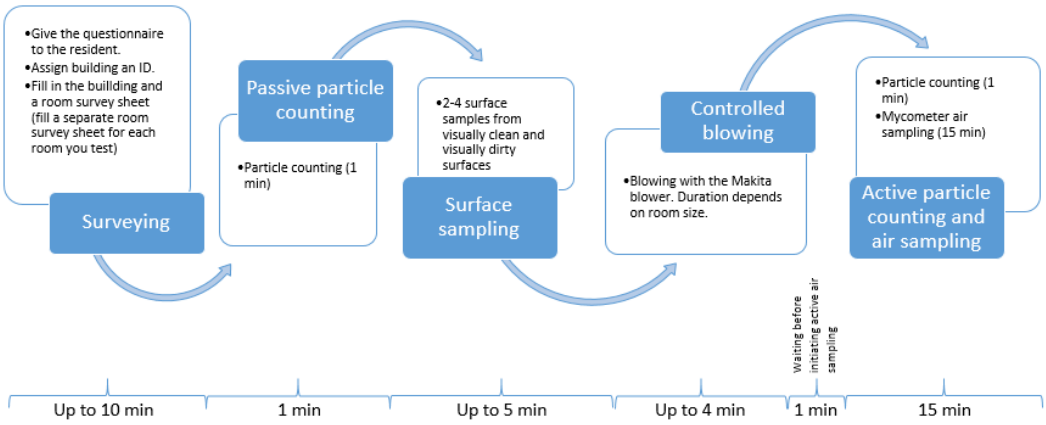


Figure 1: Extended Mycometer methodology

Individual steps of this methodology are briefly explained below.

1. SURVEYING
    a. QUESTIONNAIRE: A questionnaire was developed to collect information about, amongst other things, the residents' usage of the property. The questionnaire included 35 questions aiming at a better understanding of the usage related features of the tested space (such as the usage intensity, number of occupants including pets, if any, cleaning, heating and ventilation habits) and the residents' health conditions and overall perception of mould.
    b. PHYSICAL SURVEY OF THE PROPERTIES: This was done via two survey sheets, developed as part of this study to collect information about the physical characteristics of the building (e.g. construction date, building material and the level of maintenance) and the room (e.g. level of furnishing and cleanliness, when last used/ventilated, whether there is visible mould, or if the space ever suffered from escape of water).
2. PARTICLE COUNTING: Particle counting was done before and after the controlled blowing (i.e. both passively and actively) in order to determine how particle intensive the tested space was, and how passive and active measurement of this affected the results. The temperature and relative humidity at the time of testing was also recorded.
3. SURFACE SAMPLING: Surface sampling was performed in order to gain a more complete view of mould distribution, 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 2-5 from visually dusty/dirty surfaces by using a 3x3 cm adhesive template to define the swapping area. The surface sampling was done using a 3cm x 3cm adhesive sticker to standardise the sampled surface area (Figure 2).
4. CONTROLLED BLOWING: Blowing was done by means of a Makita blower with the aim of mixing the air and raising dust so as to reflect a somewhat high activity level within the room for active air sampling. Blowing duration was decided on the basis of the room size as follows: 1 min for room sizes up to 10 m^2, 2 min for room sizes up to 20 m^2, 3 min for room sizes up to 30 m^2, and 4 min for larger room sizes. The protocol included a 1 min waiting duration following the blowing, before starting the active air sampling to allow large particles to settle.
5. ACTIVE AIR SAMPLING: Air sampling included mould sampling by Mycometer method and particle counting. The sampling for Mycometer testing (based on NAHA activity) was done only in an active way using a flowrate of 15 L/min for 15 minutes, while particle counting was done for 1 minute (Figure 2).

Figure 2: (left to right) Surface sampling, swabs collected from a test room and air sampling (courtesy of Ms Yinzi Chen)

In addition, an even wider methodology was used for one of the test sites, a housing estate in North London This wider methodology also used a culture based method (Andersen sampling) both in a passive and active way in order to better study the difference between passive and active readings. Both passive and active Andersen sampling was done by using a 6-stage sampler with 90 mm Saboraud 4% glucose chloramphenicol agars. The sampling duration was 5 minutes. The plates were then incubated at around 20°C for 3 days before colonies were counted to calculate the colony forming units per m^3(CFU/m^3).

Testing equipment is shown in more detail in Figure 3.

Figure 3: (from left to right) All testing equipment, collected surface and air (both Andersen and NAHA methods) and agar plates at the end of incubation period

3.2. Description of the Tested Homes

The tested homes can be grouped in three subsets:

(1) **A housing estate in North London**: A housing estate composed of 64 living units – studio flats, 1-2 bedroom flats and 2 bedroom bungalows, all occupied by couples, and in case of 1-2 bedroom flats or the bungalows, by couples with up to 2 children. These were tested by a UCL team in July and October 2016. This subset of properties was tested by the wider methodology as explained above, which combined the extended Mycometer methodology with passive/active Andersen sampling. 80% of all tested rooms in this subset of properties did not have any visible mould, while 20% had some level of mould growth.

(2) **Homes of the volunteers from Polygon UK staff**: 17 properties in England, scattered across Huntingdonshire, Cambridgeshire, Peterborough, Leicestershire, Cambridgeshire, West Midlands, Staffordshire, Hertfordshire, Essex, Northamptonshire and Berkshire. These homes were tested in September-October 2016 by Polygon UK technicians, who were trained for the testing protocol as part of this pilot project by Mycometer and UCL. These properties were tested by the extended Mycometer methodology (without the Andersen sampling; shown in Figure 1). 80% of all tested rooms in this subset of properties did not have any visible mould, while 20% had some level of mould growth.

(3) **Homes tested by third parties**: A small number of tested properties were tested in-kind by third parties using the Mycometer methodology, and the findings were granted to this pilot research for analysis. None of these had visible mould in them.

A total of 71, 86 and 28 rooms were tested from these three subsets, respectively (for some examples see Figure 4). Therefore, the total size of sample set produced as part of this pilot project is 185 rooms.

Figure 4: Examples to tested rooms (top row: without visible mould; bottom row: with visible mould)

4. DATA ANALYSIS AND FINDINGS

The data obtained from the tested rooms within the housing state in North London (71 rooms)[30] were analysed to see (1) how measured values varied with presence of visible mould (Figure 2), in order to draw conclusions of the robustness of the used testing protocol to capture visible mould, and to draw conclusions regarding different sampling techniques and (2) how measured mould levels related to the physical properties of the tested homes and rooms.

[30] Only the data obtained from the North London housing estate was analysed here because (1) we used a wider methodology to test these properties which included passive and active Andersen sampling in addition to the extended Mycometer methodology shown in Figure 1, and (2) these properties were built and managed under the same administration, which brings a certain level of uniformity in terms of building age, materials, construction technique, condition, plan characteristics etc. This allowed us to be able to expand on the impact of passive and active sampling methods on the obtained readings, and to concentrate on a relatively small set of physical properties. However, a similar set of analyses is on the way for the entire dataset that was produced in this study. In this next round of analyses we are also going to analyse how occupants' behaviour (e.g. cleaning, ventilation and heating habits, usage intensity etc.) affects the measured mould levels.

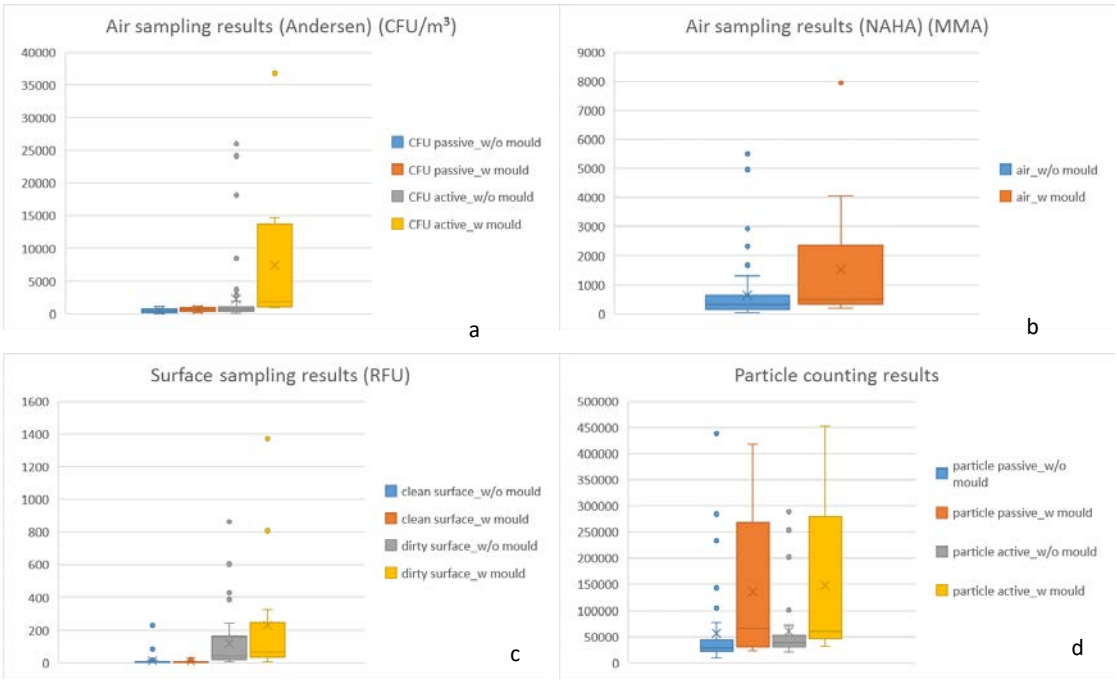


Figure 5: Summary of obtained readings as per whether there is visible mould or not within the tested spaces (a) Air mould - passive and active Andersen sampling (b) Air mould - active sampling based on the quantification of NAHA activity (c) Clean and dirty surface mould - sampling based on the quantification of NAHA activity (d) Passive and active particle counts

The data was then analysed statistically to draw conclusions on how response variables related to the physical properties of the tested rooms, collected by means of survey sheets and questionnaire[31] (Table 2). The results from this data analysis show that there is a not a single explanatory variable that has been found significant for all the response variables. Importantly, the results of passive Andersen samplings were not found to correlate to any response variables. Similarly, room cleanliness, room orientation, room window area and room size were found uncorrelated to any of the explanatory variables.

[31] Data analysis was made using R-Studio (Version 1.0.44 – © 2009-2016 RStudio, Inc.). Statistical significance was defined as *p*-value < 0.05 in all instances. Seven response variables were measured in the tested properties (71): ***Air*** (actively measured air mould concentrations quantified on the basis of NAHA activity), ***Dirty_Surface*** (average mould concentrations on visually dirty/dusty surfaces), ***Clean_Surface*** (average mould concentrations on visually clean surfaces), ***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) and ***CFU_Passive*** (number of CFUs from passively sampled air using a 6-stage Andersen sampler). It should be noted that although measurements are complete for the former 5 variables, there are some missing values for the latter two (i.e. 10% of values are missing for the *CFU_Active* and 53% values are missing for the *CFU_Passive*). The examined response variables were **home type** (flat or house), **home size** (studio, 1-bedroom or 2-bedroom), **home floor**, **home age**, **whether or not it is insulated**, **home and room orientation**, **room function**, **ceiling height**, whether or not there are **carpets/rugs**, whether or not there is **visible mould** or **other moisture induced problems** such as condensation, **temperature and relative humidity** at the time of testing, **room cleanliness**, **window area** and **room size**.

Table 2: Results of the statistical analyses to explore how various explanatory variables related to the measured response variables (X=no correlation[32], ✓: very weak correlation[33], ✓✓: weak correlation[34])

Response variables / Explanatory variables	Air (Mycometer method - active)	Average Passive Particle Count	Average Active Particle Count	Average Clean Surface	Average Dirty Surface	Active Andersen	Passive Andersen
Home Type	X	✓✓	✓✓	X	✓	X	X
Home Size	X	✓✓	✓	X	X	X	X
Home Floor	X	✓	✓	X	X	X	X
Home Orientation	X	✓✓	✓✓	X	✓✓	X	X
Home Age	X	✓✓	✓	X	X	X	X
Insulation	X	✓	✓	X	X	X	X
Room Function	X	✓	✓	X	✓	X	X
Ceiling Height	X	✓	X	X	✓	X	X
Carpets/Rugs	✓	X	X	X	✓	X	X
Visible Mould	✓	✓	✓✓	X	X	✓✓	X
Other Moisture Problems	✓	X	X	X	X	✓	X
Temperature	X	X	X	✓	X	X	X
Relative Humidity	✓	✓✓	✓✓	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

Table 2 also depicts that all linear relationships between the response and explanatory variables appear to be weak. This can be attributed, partially at least, to the small sample size, as well as to the fact that here the importance of individual explanatory variables has been explored, and not the partial contribution of multiple variables. This last observation points to the need for a multivariate analysis, which might improve the ability of multiple explanatory variables to reasonably predict the level of response variables in a given room. The number of variables that can be used in such a model, however, depends on the number of data points.

The key findings from the data analysis are listed below:

i. Passive mould testing seems to be unable to capture the visible mould presence within the tested rooms and should be replaced by an active air sampling strategy as the former can greatly underestimate the fungal reserve within a given residential indoor environment.

ii. Air sampling and surface sampling should be combined for a robust testing methodology that is able to detect localised problems and for an accurate appraisal of overall fungal agents' reserve within a given indoor environment;

iii. Lack of visible mould within a room does not necessarily mean that the air mould concentrations are low. This is because the mould can be hidden, e.g. in wall cavities, or accumulated on dirty/dusty surfaces. One should therefore not rely solely on visual inspection, and testing is necessary;

[32] i.e. p-value > 0.05
[33] i.e. p-value < 0.05 and $R^2 \leq 15\%$,
[34] i.e. p-value < 0.05 and $15\% < R^2 \leq 50\%$

iv. Visible mould (and other moisture induced problems) and actively measured air mould concentrations are related.

v. Particle counts, whether measured passively or actively, seem to be able to capture the presence of visible mould in a room;

vi. There is not a single physical characteristic of the tested rooms that seems to be significant for all measured variables. Although some of the physical features are capable of depicting trends in the data, the obtained correlations were not very strong, and these conclusions should be revisited using a larger dataset.

5. BENCHMARKING

In this study a total of 185 air samples and 438 (visually clean) and 452 (visually dirty) surface samples were collected from 185 rooms, some with and some without visible mould[35]. In this part of the project, only samples from **non-water damaged properties with no visible mould** were used to achieve benchmarking by means of a methodology previously adopted by the Danish Building and Urban Research Institute[36], based on studying the distribution of the data. The aim was to define what background levels of mould should be expected by surveyors and remediation companies in non-water damaged indoor environments without any visible mould.

The selection criteria are explained in detail in the subsequent sections.

5.1. Surface Benchmarking

The aim of studying surface mould levels in non-water damaged properties with no visible mould was to establish criteria for when a surface is to be considered completely clean and when it is in need of further examination.

5.1.1. Benchmark for when a surface is to be considered clean

The first question we try to answer is: If a surface containing mould growth is cleaned/remediated, what resulting level of mould should be used as a clear success criteria? The aim is to determine this normal background level, defined as the level of mould found in non-water damaged properties on visually clean surfaces free of mould growth.

As a "visually clean" surface is not an objective and clear cut definition, two additional examinations were introduced in order to further filter the data to be used for benchmarking. To avoid samples being taken from surfaces that were not completely clean, swab samples were visually examined before they were analysed. Only swabs that the analyst evaluated as being clean were considered for benchmarking[37]. Further, to eliminate samples with non-visible mould growth, samples that were accepted as being visually clean but where the mould

[35] Please note that in none of the tested rooms there was extensive mould – visible mould in this study is limited to staining mostly in the form of small spots and around the windows or on the window frames. Testing in mouldier properties should be pursued in the future to examine how different levels of mouldiness reflect into the readings.

[36] Surface Benchmarking: Statens Byggeforskningsinstitut (2003) Undersøgelse og vurdering af fugt og skimmelsvampe i bygninger (Study and Assessment of Moisture and Mould in Buildings): By og Byg Anvisning 204; Statens Byggeforskningsinstitut (2003) Renovering af Bygninger med Skimmelsvampevækst (Renovation of Buildings with Moulds): By og Byg Anvisning 205. Air benchmarking will be integrated into the 2018 edition.

[37] Surface swabs were categorised based on their cleanliness from 0 to 3, from the cleanest to the dirtiest. This exercise was done by the same analyst to maximise consistency, and only the cleanest swabs were used for the clean surface benchmarking.

analysis showed a heightened NAHA activity were also analysed by microscopy [38]. If hyphae, conidiophores or high proportion of mould spores were found (indicating a non-visual mould source), the sample was discarded.

As such, a total of **259** samples coming from **visually clean surfaces** within **non-water damaged rooms with no visible mould** that were **found not to include any mould growth** by microscopy were used for benchmarking when a surface is to be considered free of mould growth. An analysis of the distribution of this clean surface data shows that approximately 98% of the visually clean surfaces are below 25 RFU[39] (Figure 3). Therefore, we suggest that a mould level ≤25 RFU could be targeted by any remediation work on a contaminated surface as success criteria.

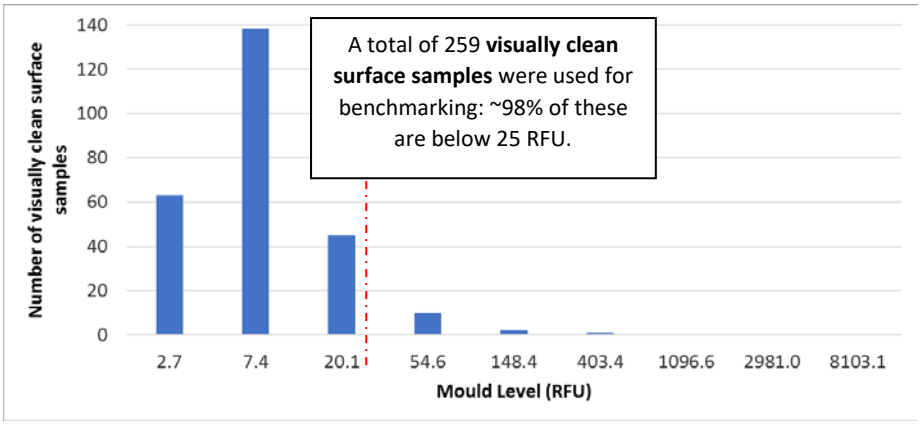


Figure 6: The level of measured NAHA activity on uncontaminated, visually clean surfaces within rooms with no visible mould problem

5.1.2. Benchmark for when a surface is to be considered in need of further examination

Indoor mould problems differ from other indoor air quality issues in that mould is present in all buildings, and even non-problem properties with no building related sources of mould contain various levels of mould on the surfaces depending on, for example, the amount of accumulated particles on them, i.e. their levels of cleaning. But the point at which surface mould concentration goes beyond this "normal" presence of mould due to dust and dirt, and indicates that the surface might be in need of further examination and possibly remediation is not clear. As highlighted also by the latest Guideline on Assessment and Remediation of Fungi in Indoor Environments released by the New York City Department of Health in 2008, from a remediation point of view, a contaminated surface can be defined as one that contains mould growth, either visible or non-visible (i.e. germination and formation of hyphae). Mould growth therefore is the relevant physical phenomenon to define the term "mould problem", and the technologies used for measuring mould should be able to distinguish between naturally occurring levels on dirty/dusty surfaces and surfaces with mould growth. The only way to detect mould growth is through measurement - a surface that contains mould growth has significantly higher concentrations of mould than a surface that contains spores accumulated in dust, and one can understand if this level is exceeded by comparing the measured value against typical mould levels in non-water damaged buildings with no visible mould or mould growth.

[38] After the NAHA activity was determined, 1 ml of sterile demineralized water was added to the filter. The filter (still situated in the filtration chamber) was then vortex mixed for 30 seconds to release mould from the filter surface and a sample was then taken for microscopy. The microscopy evaluation of the sample was always performed by the same analyst.

[39] The MMA (Mycometer Air Value) is measured in Relative Fluorescence Units (RFU) and is a measure of the NAHA (β-N-acethylhexosaminidase) activity obtained when following the Mycometer protocol for sampling and analysis.

Also here, samples with heightened NAHA activity were further examined by microscopy, and those that had hyphae, conidiophores or high proportion of mould spores were eliminated from this process. As such, a total of **298** samples coming from **visually dirty/dusty surfaces** within **non-water damaged rooms with no visible mould** that were **found not to include any mould growth** by microscopy were used here for benchmarking when the mould levels on a surface exceed what can be safely considered non-problem and in need of further examination. An analysis of the distribution of this dirty surface data shows that approximately 98% of the visually dirty surfaces are below 450 RFU (Figure 4, blue columns). Therefore, 450 RFU could be taken as the threshold for a surface to be accepted to be free of mould growth despite a somewhat heightened level of NAHA activity due to dust/dirt accumulation.

41 dirty/dusty surfaces within rooms with or without visible mould that were established by microscopy to have mould growth are also shown in Figure 4 as red columns **for comparison purposes only.**

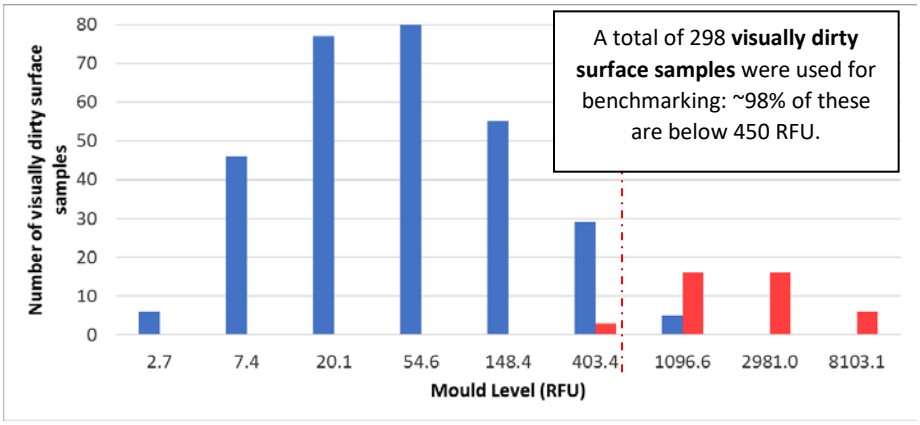


*Figure 7: The level of measured NAHA activity on uncontaminated, visually dirty/dusty surfaces within rooms with no visible mould problem (blue columns); Red columns indicate results obtained from samples that were established by microscopy that had mould growth – these are shown for demonstration purposes only and **were not used for benchmarking**.*

Our suggested benchmark categories for surface samples are given in Table 3.

Table 3: Suggested categories for surface mould concentrations

A NAHA activity on surfaces 0-25 RFU	This level of mould concentration is found on non-problem, visually clean surfaces. This category could be targeted as a result of a surface remediation.
B NAHA activity on surfaces 26-450 RFU	This level of mould is higher than that typically found on visually clean surfaces, and represents the level measured on non-problem dusty or dirty surfaces.
C NAHA activity on surfaces > 450 RFU	This level of mould indicates surfaces with concentrations beyond we typically found on dusty or dirty surfaces in non-problem rooms. Future work should follow to further explore this range to study various levels of mould growth.

5.2. Air Benchmarking

The aim of studying airborne levels of mould was to establish criteria for 1) when mould remediation work can be seen as successfully performed and 2) when the airborne mould concentrations are above what is normally found in non-water damaged properties with no visual mould.

Similar to the approach used for surface samples, all air samples to be used for benchmarking were taken from non-water damaged properties with no visual mould growth. However, because mould growth is not always visible, samples with high NAHA activity were further examined by microscopy. If the microscopy showed hyphae, conidiophores or high numbers of mould spores (indicating a non-visible mould source) the sample was discarded from the dataset to be used for benchmarking.

As such, a total of **130** air readings from **non-water damaged rooms with no visible mould** that were **found not to include any mould growth** by microscopy were used for air benchmarking (Figure 6, blue columns). An analysis of the distribution of the data obtained from rooms with no visible mould shows that approximately 98% of the air samples are below 1700 RFU. Therefore, values above 1700 RFU might be indicative of the presence of a mould source and a need for further examination.

A smaller number of samples were taken from properties where there was visible mould growth or from a few properties with no visible growth but where both NAHA measurements and microscopy revealed high levels and hyphae, conidiophores or very high number of spores (indicating a hidden mould source). **For comparison purposes only**, the results of these 10 samples are shown as red columns in Figure 6.

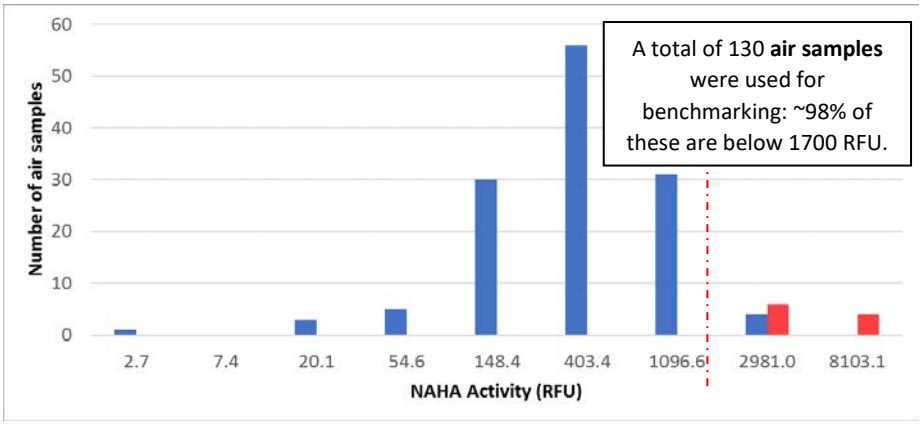


*Figure 8: The level of measured NAHA activity in the air within rooms with no visible damp or mould problem (blue columns). Red columns indicate results obtained from samples that were established by microscopy that had mould growth – these are shown for demonstration purposes only and **were not used for benchmarking**.*

The air values lower than 1700 RFU were further divided into a set of suggestive categories shown in Figure 6 (bottom). Category A+ corresponds to approximately lowest **one third** of all readings; category A corresponds to readings between 33% and **90%**; category B corresponds to readings between 90% and **98%**. Category C represents around **2%** of the rooms with the highest level of mould in the air[40].

Based on this, our suggested benchmark categories for air samples are given in Table 4.

[40] The 33% and 90% cut-off points were chosen on the basis of a visual assessment of the distribution of our data, and are only suggestive.

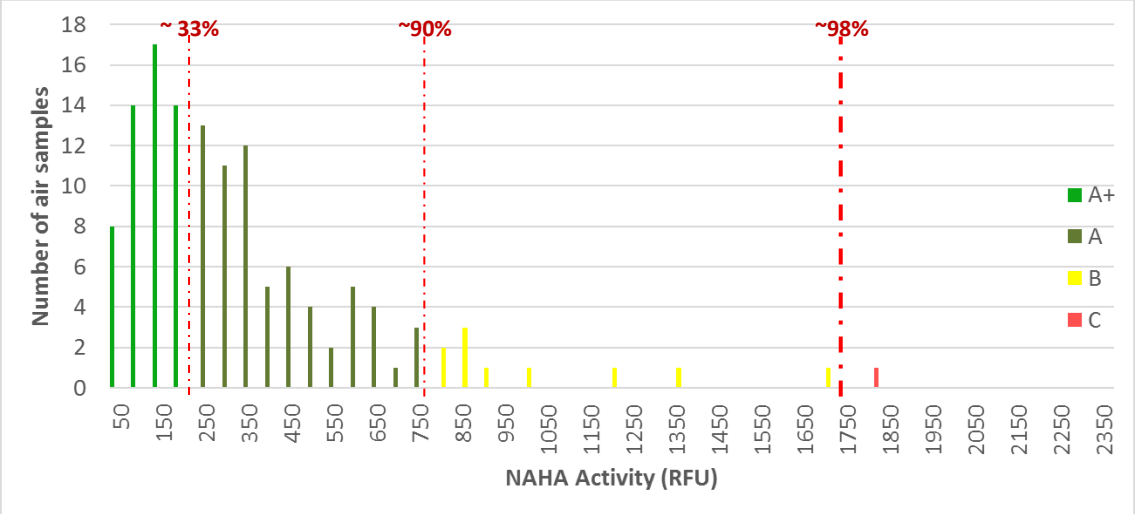


Figure 9: Suggested benchmark categories

Table 4: Suggested categories for air mould concentrations

A+ NAHA activity ≤ 200 RFU	This level of air mould concentration is typically found in rooms with no visible mould, with a high cleaning standard. This category could be targeted following a remediation.
A NAHA activity 201-750 RFU	This level of mould in the air can be understood as the level found in non-problem rooms, typically with a "normal" cleaning standard.
B NAHA activity 751-1700 RFU	This level of mould is higher than the level that is typically found in non-problem rooms with normal cleaning standard. If encountered in a room with very high cleaning standard, this category means that the room may need further investigation.
C NAHA activity > 1700	This level of mould is higher than what we found in non-problem rooms with no mould growth. This may be due to the presence of a mould source in the room. Future work should follow to further explore this range to study various levels of mouldiness.

The key findings from this component of our research are summarised below:

i. For surface sampling, values higher than 450 RFU indicate that the surface could contain mould growth, and needs to be further examined;

ii. The success criteria for mould remediation on the surfaces could be 25 RFU or lower;

iii. For air sampling results we suggest a four category classification system: A+ ≤ 200 RFU, A ≤ 750 RFU, B ≤ 1700 RFU and C > 1700 RFU, (A+, A, B and C being representative of the spectrum from least to most mouldy air classes). 200 RFU could be used as the benchmark of an acceptable air mould level after a successful mould remediation;

iv. The herein suggested surface benchmarks are closely comparable with the ones established for Denmark, currently in use by the Danish Building Research Institute;

v. The herein suggested benchmarks can only be used when following the exact sampling and analysis protocols used in this study, and only for residential properties;

vi. Future work should revisit these suggestions using larger datasets, and should also explore further categorisation of various levels of mouldiness.

6. DISCUSSION AND FUTURE WORK

A brief discussion of the findings is given below:

The methodology used here was found able to capture the presence of visible mould and other indicators of possible moisture induced problems (such as condensation, musty smell etc.). It was shown that benchmarking is possible if one adheres to a testing protocol robust enough to spot a visible mould problem. The bias of passive readings was also demonstrated clearly.

The benchmarks suggested here were determined on the basis of whether or not there is visible mould within the tested rooms, and they do not account for mould that is hidden in cavities or elsewhere. Similarly, they should be referred to only when the exact same testing protocol is used and strictly for residential properties.

There is a large level of uncertainty associated with the data because of the complexity of the problem at hand, and also due to limited amount of data. As part of this pilot project, we have analysed data obtained from buildings from a relatively small portion of the UK, which may not be representative for either the UK building stock as a whole, or all local climate regimes within the UK. Similarly, the herein suggested benchmarks currently may not be representative of the entire building stock in the UK. The fact that they are very similar to the ones developed for Denmark suggests that the local climate regimes may not be as influential on the findings, however this should be further tested by future studies.

Importantly, this study did not include species identification. Seasonal variation, which is another reportedly important parameter, has not been considered.

Based on our findings and the discussion given above, we think that the future work should be carried out in consideration of the points below:

1. Future studies should be undertaken to validate (or disprove) the conclusions drawn here using a larger dataset, including also homes with various levels of mouldiness. This is important in order also to further explore on the impact of different constructive features on mould susceptibility.
2. Other methods of assessment should also be tested where they are considered viable and robust. However, the aim of this testing will be eventually to reduce the number of tests to the minimum number required to give robust information and benchmarking. It is important to establish a national or even global protocol for mould testing and benchmarking so that results reported from different studies can be properly compared.
3. Our findings suggest that particle counts can be a good indicator of visible mould in a given indoor environment. If the relationship between particle counts and mould levels is further established by future studies, the benchmarking efforts should be extended to particle counts.
4. Whatever methodology is finally agreed should be used, along with the resultant information and benchmarking, to take forward research into the links between buildings, mould and human health, as suggested in the UKCMB report on Health and Moisture in Buildings.
5. The extent and nature of mould problem induced by escape of water and flooding/wind-driven rain should be investigated by field and lab tests.
6. Mould is a widespread concern in a number of other sectors, and research efforts such as this should be extended to cover workplaces and heritage buildings.
7. More data analysis will soon follow in order to explore the impact occupant behaviour (e.g. levels of occupancy, cleaning, laundry and ventilation habits, cooking and bathing) on the measured mould levels.

For further information please contact UKCMB.