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Normal background levels of air and surface mould reserve in UK residential building stock

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ABSTRACT

This paper reports results obtained from a surface (both visually clean and dirty/dusty surfaces) and active (aggressive) air testing scheme on 140 residential rooms in England, without visible water damage or mould growth, along with a few rooms with visible mould growth/water damage tested for comparison purposes, with the aim of providing background levels of mould in non-water-damaged interiors to benchmark a "normal" indoor environment, and in turn when there is a need for further investigation, and, possibly, remediation. Air and surface mould was quantified based on the activity of β-N-acetylhexosaminidase (EC 3.2.1.52; NAHA). The obtained readings showed a log-normal distribution. 98% of the samples obtained from visually clean surfaces were equal to or less than 25 relative fluorescence units (RFU), which is suggested to be the higher bound for the range which can be used as a success criterion for surface cleaning/remediation in non-problem buildings. Of samples obtained from visually dirty/dusty surfaces, around 98% were below 450 RFU, which is suggested to define the lower-bound for abnormally high levels of mould, rare even on dirty/dusty surfaces. Similarly, around 98% of the air samples were found to have 1700 RFU or below. Values above 1700 RFU are therefore unlikely in a non-problem indoor environment and can be indicative of a possible problem inducing mould growth. The samples with values below 1700 were further divided into three proposed sub-categories. Finally, these values were compared to those obtained in Denmark in a similar study and are currently used in national standards, and they were found highly congruent, suggesting that local climate regimes and room functions might not be as influential on indoor mould levels, or that the nuances between UK and Denmark in terms of these factors are not strong enough to lead to sizable changes in the typical indoor mould levels in these countries.

Keywords: mould; surface sampling; active (aggressive) air sampling; NAHA; UK; Denmark

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1. INTRODUCTION

Mould growth is one of the most persistent problems affecting the indoor built environment and the UK is among those countries where it is claimed that this problem is especially pronounced (May et al., 2017). However, despite the increasing needs of the remediation and insurance industries for clear definitions, methodologies and benchmarks, indoor mould growth in buildings is still to large extent an area of confusion for academics and practitioners alike. The interpretation of any obtained results is difficult because there is no scientifically established criteria on the acceptable mould levels or compositions, or on the baseline levels for "normal" indoor environments (HUD, 2005; Gots et al., 2003). An already limited number of suggested values for acceptable indoor mould levels are based on institutional consensus or professional judgement rather than testing data obtained by means of well-established, transparent testing protocols, and are often contradictory.

The differentiation between a "problem" indoor environment and a "normal" indoor environment has been the subject of many scholarly publications. Richards (1954), in his early review, defines a damp/problem home as one where there is visible mould, which is in broad agreement with the findings of Hyvärinen et al. (1993). On the other hand, it has been shown that the lack of visible mould does not necessarily mean that mould is not present and that testing is therefore essential (Aktas et al., 2018 a&b). However even when data is available via testing, guidance as to when mould levels should be taken as indicative of a need for further investigation and remediation is still rather superficial. The guidelines either have no specific criteria, or use visible mould growth (or water damage, or musty odours) as the remediation triggering event (e.g. AIHA, 2013; NY City Department of Health and Mental Hygiene, 2000), which are of little use when the high mould concentrations have not (yet) manifested in the form of visible mould, or when the mould is hidden inside cavities. Within this framework, this study aims to establish normal background levels of mould in normal, non-water-damaged buildings without visible mould in order to improve the decision-making process as to when a given indoor environment is in need for remediation.

To this end, a testing protocol previously reported by Aktas et al. (2018a&b) was used, which showed that surface and air sampling should be combined to identify local mould problems, and that an active (aggressive) air sampling strategy, i.e. sampling actively mixed air, better represent indoor mould concentrations than a passive sampling approach, i.e. sampling still air. In this study, the surface and air mould concentration values were measured by means of the quantification of N-acetylhexosaminidase (EC 3.2.1.52; NAHA) activity, which depicts all fungal propagules (spores, hyphae) (Reeslev and Miller, 2000) and microfragments (Adhikan et al., 2013). This method has been previously 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, 2003 a&b), as well as ASTM 7338-10 (2014).

2. MATERIALS AND METHODS

In this study, a total of 140 non-water-damaged rooms with no visible mould were tested to identify normal background levels of mould and to benchmark when a surface or indoor air needs remediation. To this end, 140

air samples and 629 surface samples were collected (313 from visually clean surfaces and 316 visually dirty/dusty surfaces).

Study site: The buildings used for this study include houses (17%), flats (59%), and bungalows (24%) of different ages (pre-1945: 3%, 1946-1970: 70%, 1971-1990: 24%, 1991-today: 3%), and different materials and construction techniques (solid brick masonry, brick masonry with cavity walls with and without insulation, concrete) selected from London and the surrounding counties: Huntingdonshire, Cambridgeshire, Soke of Peterborough, Leicestershire, Cambridgeshire, the West Midlands, Staffordshire, Hertfordshire, Essex, Northamptonshire, and Berkshire. In these buildings a total of 140 non-water-damaged rooms with no visible mould were tested using a testing protocol composed of surface sampling and active (aggressive) air sampling as detailed below.

Surface sampling: Surface samples were taken using a sterile swab wetted in a sterile saline to enhance swabbing efficacy. A 3×3 cm adhesive template was used to demarcate the swabbing area. In each room, 3-5 samples were taken from both visually clean and visually dirty/dusty (but not visibly mouldy) surfaces.

Active (aggressive) air sampling: Once the air sampling equipment was set up in the room, a handheld blower (Makita BUB 182, 18V, 0.043m3/s, Makita Corporation, Anjo, Aichi, Japan) was used to disturb all surfaces in the room twice from a distance of approximately 2m using maximum power on the blower. Air sampling was started 1 minute after the blowing phase (to allow large particles to settle), using a flow rate of 15 LPM for 15 minutes (225 litres total volume), through a cassette preloaded with a 25 mm, 0.8 µm pore size Mixed Cellulose Ester filter (Zefon International, Inc. Ocala, FL, USA). The filters were placed 1.5 m above the ground with the open face filter pointing upwards.

Mould quantification: Mould concentrations in both surface and air samples were quantified by measuring the activity of β-N-acetylhexosaminidase (NAHA) according to a standardized protocol (Mycometer A/S, Denmark). Firstly, an enzyme substrate containing 4-methyl umbelliferyl was added to the filter or swab samples. After around 30 minutes of reaction time (depending on ambient temperature), the resulting fluorescence was measured in relative fluorescence units (RFU) using a manual fluorometer (Turner Design US/Mycometer version), and the substrate blank value subtracted. One RFU is equal to 33.3×10^{-2} pmol 4-MU/ml reaction volume/min. The sampling area for the surface samples was 9 cm^2 and the reaction volume used for analysing surface samples was 2 ml. The sample volume for air samples were 225 l and the reaction volume for air samples was 1 ml.

Sample Filtering: The obtained air and surface samples were then filtered using visual assessments and microscopy analysis, as described below.

a. Visual assessment: Because surface sampling was done from both visually clean and dirty/dusty surfaces and because there is not an objective definition for a clean surface, an additional visual assessment step was carried out to categorise the clean surface samples based on their cleanliness from 0 to 3 from the cleanest to the dirtiest. Only cleanest swabs (category 0, i.e. no stain or discolouration at all) were used for benchmarking when

a surface is to be considered clean. The visual assessment was carried out by the same analyst throughout in order to ensure consistency.

b. Microscopy analysis: Although all samples were taken from non-water-damaged rooms with no visible mould, because mould growth is not always visible, all surface and air samples which gave a heightened NAHA activity reading were examined under microscopy. To this end, 1 ml of sterile demineralized water was added to the filter after NAHA activity was determined. 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 analysis of the sample was performed by the same analyst throughout in order to ensure consistency. All samples that were found to contain hyphae, conidiophores, or high proportion of mould spores were eliminated from the benchmarking process. In cases where the high NAHA readings were found to be associated with non-mould origin (e.g. skin cells, different types of fibres, and pollen with little mould spores, and other debris due to poor cleaning standard), the samples were included in the benchmarking.

3. RESULTS AND DISCUSSION

Surface sampling results: The identification of normal background surface mould concentrations was undertaken in two stages: (1) surface samples taken from visually clean surfaces were used to benchmark the upper threshold for what level of mould concentration to target following cleaning/remediation work, and (2) surface samples taken from visually dusty/dirty surfaces were used to establish the lower threshold for when a surface is likely to have mould growth, and therefore should be considered for remediation.

In order to identify when a surface is to be considered clean, firstly 313 samples obtained from visually clean surfaces were filtered through visual assessment and microscopy as explained in Materials and Methods. All samples that were not found perfectly clean or were established via microscopy that contained mould growth (n=54) were eliminated, and the remaining 259 samples were used for benchmarking.

In the next stage, in order to identify when a surface is to be considered in need of remediation, only visually dirty/dusty surfaces obtained from rooms with no visible mould growth were used. Also in this case, samples containing mould growth were filtered through microscopy analysis and samples that were found to contain mould growth (n=18) were eliminated from 316 samples. The remaining 298 from visually dirty/dusty surfaces that were found not to include any mould growth were used for benchmarking.

The distribution of the readings from these 259 and 298 samples respectively from the visually clean and dusty/dirty surfaces with no mould growth (blue columns) is shown in **Error! Reference source not found.**, along with the readings obtained from 164 and 167 samples with no mould growth collected using the same testing protocol within a total of 17 properties in Denmark for a similar study (grey columns). The data show that around 98% of all samples from clean surfaces are below 25 RFU, and around 98% of all samples from dusty/dirty surfaces are below 450 RFU, both for the UK and Denmark. In addition, results from an additional 18 readings that were established by microscopy analysis to have mould growth are also shown in red columns for comparison purposes.

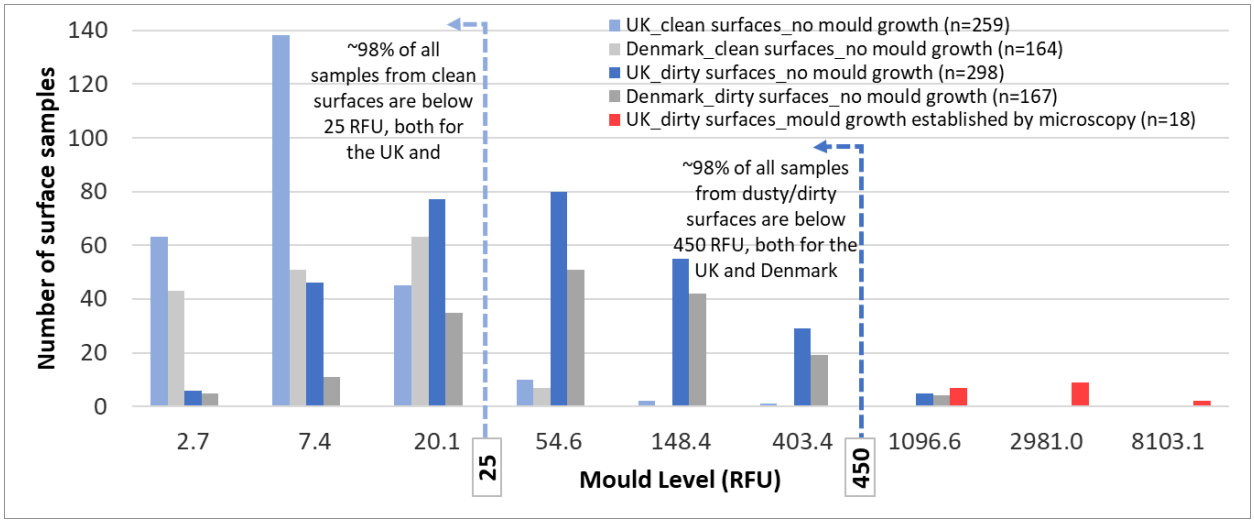

Figure 1: Surface mould levels

Some of the important conclusions and suggestions based on these observations are summarised below:

(1) For the set of buildings tested here, 25 RFU is the higher bound for a visually clean surface from a room with no visible mould growth or water damage, and could be used as a success criterion for cleaning and remediation.

(2) NAHA activities above 450 RFU are most likely beyond the naturally occurring levels of accumulated mould propagules found on dusty or dirty surfaces in rooms with no visible mould growth or water damage. 450 RFU is therefore the lower bound for a visually dusty/dirty surface from a room with no visible mould growth, and could be used to flag need for further investigation.

(3) There is a clear differentiation between samples obtained from surfaces with no mould growth (blue and grey columns), and those that were established to have mould growth via microscopy (red columns). This zone beyond 450 RFU should be further examined by future research to study the distribution of data obtained from indoor environments with different levels of mouldiness.

(4) The surface benchmark values that we identified here are highly comparable to those currently in use by the Danish Building Research Institute for more than 15 years as part of the Danish Mould Guidelines (Statens Byggeforskningsinstitut, 2003a&b). A benchmark of NAHA activity levels ≤25 RFU (referred to as Category A by the Danish Building Research Institute; see Statens Byggeforskningsinstitut, 2003a) has been used as a success criterion for the cleaning/remediation of surfaces containing mould growth. Similarly, readings >450 RFU (defined as Category C, *ibid.*) is accepted as the criterion to define when to remediate a surface. Category B defined as readings between 26 and 450 RFU, on the other hand, comprises typical mould concentration levels on dusty/dirty surfaces without mould growth (*ibid.*).

Air sampling results: For the purposes of benchmarking normal background air mould levels, a similar approach was adopted. Of the total 140 air samples collected, all that demonstrated a heightened NAHA activity were examined via microscopy and eliminated if they showed hyphae, conidiophores or very high spore counts (n=10). The remaining 130 air readings were used for air benchmarking.

The distribution of the readings from these 130 samples (blue columns) is shown in Figure 2 along with the readings obtained from 86 air samples taken during a similar study from 12 properties in Denmark (grey columns). The data show that around 98% of all air readings were below 1700 RFU, both for the UK and Denmark. In addition, results from 10 samples which were established by microscopy that did have mould growth are shown in red.

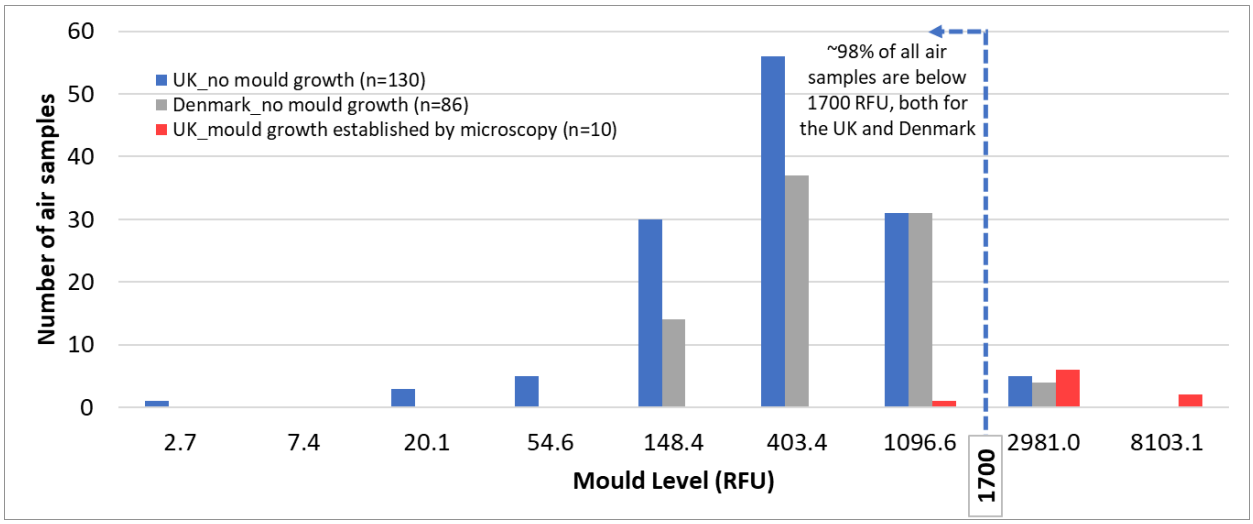

Figure 2: Air mould levels

Based on these data we suggest that air values higher than 1700 RFU are most likely indicative of mould growth and might be considered for further examination, and potentially for remediation. Air values lower than 1700 RFU can be further divided into 3 suggestive categories:

(1) The lowest one third of all readings (≤200 RFU) could work as the success criteria after a cleaning/mould remediation work.

(2) 33% to 90% of all readings (201-750 RFU) could be considered the normal background levels of mould within rooms with a good to normal cleaning standard.

(3) 90% to 98% of all readings (751-1700 RFU) could indicate rooms with a poor to bad cleaning standard, therefore if a reading within this range is obtained from a room with a high cleaning standard, the room should be checked thoroughly for a possible hidden mould source.

(4) Only around 2% of the all readings were >1700 RFU, meaning this is an unlikely outcome from indoor environments with no water damage or visual mould growth, indicating potential for mould growth from an indirect source (hidden mould growth e.g. in building cavities), or an extremely poor cleaning standard.

The air benchmarks have not yet penetrated into the guidelines by Danish Building Research Institute, but there are plans in that direction, including the above-mentioned 3 categories, which may be slightly different in the Danish case.

4. DISCUSSION

In this study, the normal, background levels of mould on the surfaces and in the air were established using samples obtained from 140 rooms from homes scattered across London and surrounding counties, tested for the activity of β-N-acetylhexosaminidase (EC 3.2.1.52; NAHA). The obtained benchmarks as well as the distribution of the data were found highly comparable to those obtained from a set of Danish properties. There are two important observations that can be made based on this extremely high similarity:

(1) Earlier work suggests that the mould levels within a given indoor environment is, among many other factors, a product of prevalent outdoor climatic conditions (e.g. for a species focussed discourse see Amend et al., 2010) and how the indoor environment is used, i.e. lifestyle characteristics including heating, cleaning and ventilation habits (e.g. Chew et al., 2003; Ren et al., 2001). While the extent of these relations are far from conclusive, the stark similarity between the values obtained for English and Danish properties may suggest that these might not be as important as initially thought, or at least that the nuances between UK and Denmark in terms of these factors are not strong enough to lead to sizable changes in the typical indoor mould levels in these countries (cf. Haverinen-Shaughnessy, 2012). This should however be further examined by future research.

(2) While all properties tested in the UK are strictly residential, the data used for the Danish study were obtained from a number of non-residential properties in Copenhagen, including the Geology Museum, Zoology Museum, University of Copenhagen Pharmaceutical College, Institutes of Zoology, Botanic, and Molecular Biology, as well as schools and a hospital. That these data yielded highly comparable results to the UK suggest that the function of the indoor environment does not play an important role in the typical indoor mould levels. This should however be further examined by future research.

5. CONCLUSIONS

The lack of reproducibility is one of the major issues in mould research and is mainly due to a lack of standardisation of sampling equipment, sampling protocols, and analysis methods. However even when all these is standardised, it is not easy to interpret the obtained readings. In this study, a standard mould measurement method based on the quantification of the activity of N-acetylhexosaminidase (NAHA) was used for surface and active (aggressive) air testing. The distribution of surface and air readings that were obtained from non-water-damaged rooms with no visible mould and no mould growth was studied in order to define normal background levels of mould and benchmark when a surface/indoor environment should be considered clean, and when it should be considered in need for further examination, and potentially remediation.

Our suggested benchmarks are as follows:

- Surface

  0-25 RFU: This level of mould concentration is found on visually clean surfaces within non-water-damaged indoor environments, and could be targeted following a surface cleaning/remediation.

  26-450 RFU: This level of mould is higher than levels typically found on visually clean surfaces, and represents the level measured on dusty/dirty surfaces with no mould growth.

  > 450 RFU: This level of mould indicates surfaces with concentrations higher than levels typically found on dusty/dirty surfaces in non-problem indoor environments, and is likely to indicate mould growth. Future work should be undertaken to explore this range further and to study various levels of mould growth and contamination.

- Air

  ≤ 200 RFU: This level of air mould concentration is typically found in rooms with no visible mould, with a high cleaning standard, and could be targeted following a remediation.

  201-750 RFU: This level of mould in the air is the level found in non-problem, non-water-damaged indoor environments, typically with a normal cleaning standard.

  751-1700 RFU: This level of mould is higher than levels typically found in non-problem rooms with normal cleaning standard. If encountered in a room with very high cleaning standard, this category may mean that the room is in need for further investigation.

  > 1700 RFU: This level of mould is higher than levels typically found in non-problem indoor environments, and is likely to indicate mould growth. Future work should be undertaken to explore this range further and to study various grades of mould growth and contamination.

The benchmarks here are specific to the testing method and protocol used. However, the same approach can be adopted to any other mould testing technology with a clear testing protocol. The association of our suggested thresholds to health implications should be explored by further research, which should also include speciation, and different levels of mould growth and contamination.

Finally, the similarity of benchmarks defined for the UK and Denmark may suggest that the some of the factors that are currently accepted to govern typical indoor mould levels - primarily the climatic conditions and lifestyle - are either not as influential, or there are not sufficiently different in these two countries to lead to a substantial difference in the findings.

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