

CONCLUSIONS ON HEALTH IMPLICATIONS OF AIRBORNE MOLDS: ANALYSIS OF AIRBORNE MOLDS IN 11 CONTAMINATED HOUSES USING A NEW METHOD OF EVALUATION

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ABSTRACT

Airborne molds in 11 contaminated houses were investigated using a new method of evaluation.

There was a fluctuation of colony forming units/m³ from < 1% to > 90% when compared to the levels of total cell count method (tcc). The results of the outdoor samples are always higher as the indoor samples.

The investigation with a cfu/tcc evaluation showed the influence of time on the results in 5 ways:

In an older microflora, many molds have not survived the elapsed period of time and can because of this not be confirmed by the determination of colony forming units/m³ (cfu/m³). Older mold sources emit more spores and mycelia parts to the air as younger sources. Through an air flow close to the contaminated surface of the building material the mold spores and fragments will get airborne and these microorganisms will show the same age as those on the building material. The comparison of cfu/tcc can be used as an indication of the age of the source of the airborne contamination with molds (cfu/tcc-evaluation). Outdoor and indoor samples are seldom of the same age and this indicates that the indoor and outdoor sources of airborne molds have come from different places and should consequently not be used as reference measurements.

Out of 29 measurements of just cfu/m³ there were no information indicating an indoor mold source. When additional methods of confirmation were used (cfu/tcc-evaluation) there were 18 samples indicating indoor mold sources.

The toxic and allergenic potential of airborne molds is dependent on the amount of biomass/m³ or on the total number of microorganisms/m³ and independent of the number of colony forming units/m³ (cfu/m³). In conclusion with all results of this investigation an attempt to correlate the number of airborne colony forming units to any types of health problems is irrelevant.

Introduction/ Goal of study

Increased awareness of the danger to human health caused by airborne molds in housing has triggered an interest in reliable methods of determining a potential

mold exposure. In many publications, the correlation between living in damp houses and negative health effects could be established (Bornehag et al., 2001; Bornehag et al., 2004; Fisk, Lei-Gomez and Mendell, 2007; Institute of Medicine, 2004; Mudarri and Fisk, 2007; WHO Guidelines for Indoor Air Quality, 2009; Tischer, Chen and Heinrich, 2011; Norbäck, 2001) but the correlation with the colony forming units (cfu/m³) of airborne mold could not (WHO Guidelines for Indoor Air Quality, 2009; Malmberg et al., 1986; Malmberg et al., Norbäck et al., 1993). By the risk assessment of airborne molds, with emphasis on the airborne colony forming units (cfu) as the cause of illness, the standard method of detection is the measurement of cfu/m³ air.

Molds grow only if the relative humidity of the surroundings is high enough to support it (> 70%). When for example building materials dry out after the cause of the water damage has been repaired, the relative humidity is lowered and the mold growth stops. With time more and more molds on the material are so inhibited in their metabolism that they can not even start to grow as cfu on the nutrient rich agar media in the labs. In this low activity status the mold can not be found with laboratory methods based on plate count techniques, they can just be detected with methods which count the mold although they do not grow (for examples microscopic counting).

The diminishing capacity of the mold to grow over time (and be detected as cfu) in combination with a constant remaining total cell count, give microbiologists the possibility to estimate how old a special surface contamination could be.

The aim of this investigation is to test the hypothesis that airborne microorganisms from contaminated surfaces will have a different age pattern between different rooms and houses as well as when compared to microorganisms in air samples from outside the building.

METHOD

Air samples, were initiated in damp houses where the presence of a high concentration of a mold growth on surfaces already had been proven. The evening before the sampling day all houses were ventilated through windows for > half an hour and then the rooms were locked and reopened at the time for sampling the next day.

The sampling was made with the filtration method (Camnea method). Polycarbonate filters with a pore size of 0.4µm and a diameter of 37mm were placed on support pads in sterilized filter cassettes (Millipore). With an air flow of 2 liters per minute over 4 hours, air was sucked through the filter holder with an air sampler (SKC).

After the sampling, the filter and the cartridge were washed with a sterile washing liquid. 100 µl of the sample was dyed with acridine-orange (fluorescens dye)

and filtrated through a black polycarbonate filter. Thereafter the microorganisms on the filter surface were counted under a fluorescence microscope. In the next step, colony formed units (cfu) were plated out on three standard media, incubated at 25 °C and counted as well as differentiated after seven days.

Total cell count (tcc) of microorganisms stained with fluorochromes such as acridine orange has been used for a long time in different environments, such as marine research (Zimmerman & Meier-Reil 1974; Jones & Simon 1975), rapid determination in food samples (Pettipher et al., 1980; Pettipher & Rodrigues 1982) and counting of airborne microorganisms in highly contaminated environments (Palmgren et al., 1986).

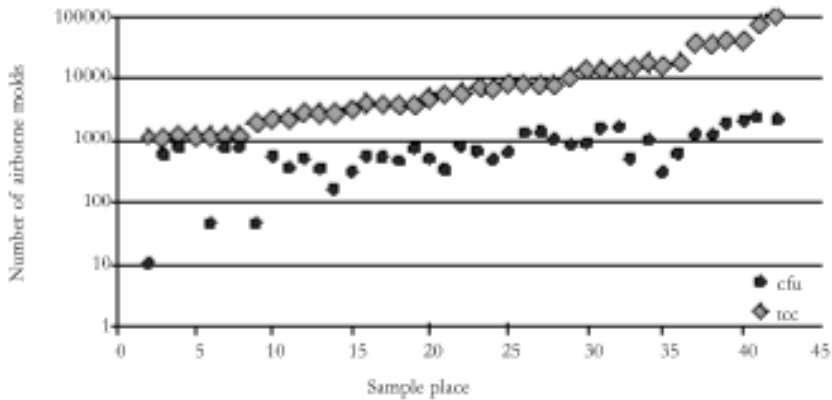
Additional staining with FDA (fluorochrome) shows the metabolic activity of microorganisms and gives the researcher information that makes a determination of the age of the mold growth on building materials possible. Labor Urbanus GmbH for example, uses this method of investigation to confirm if more than just one water damage has caused particular mold damage to a building.

In the rooms where air sampling was carried out, mold growth on wall surfaces was investigated with an adhesive tape method (mold spores and mycelia/cm²).

RESULTS

The analyzes of airborne molds show a fluctuation in the levels of cfu/m³ from < 1% to > 90% compared to the levels of airborne molds estimated with the total cell count method (fig. 1).

Fig. 1. Two methods of analyzes are compared in 11 houses.



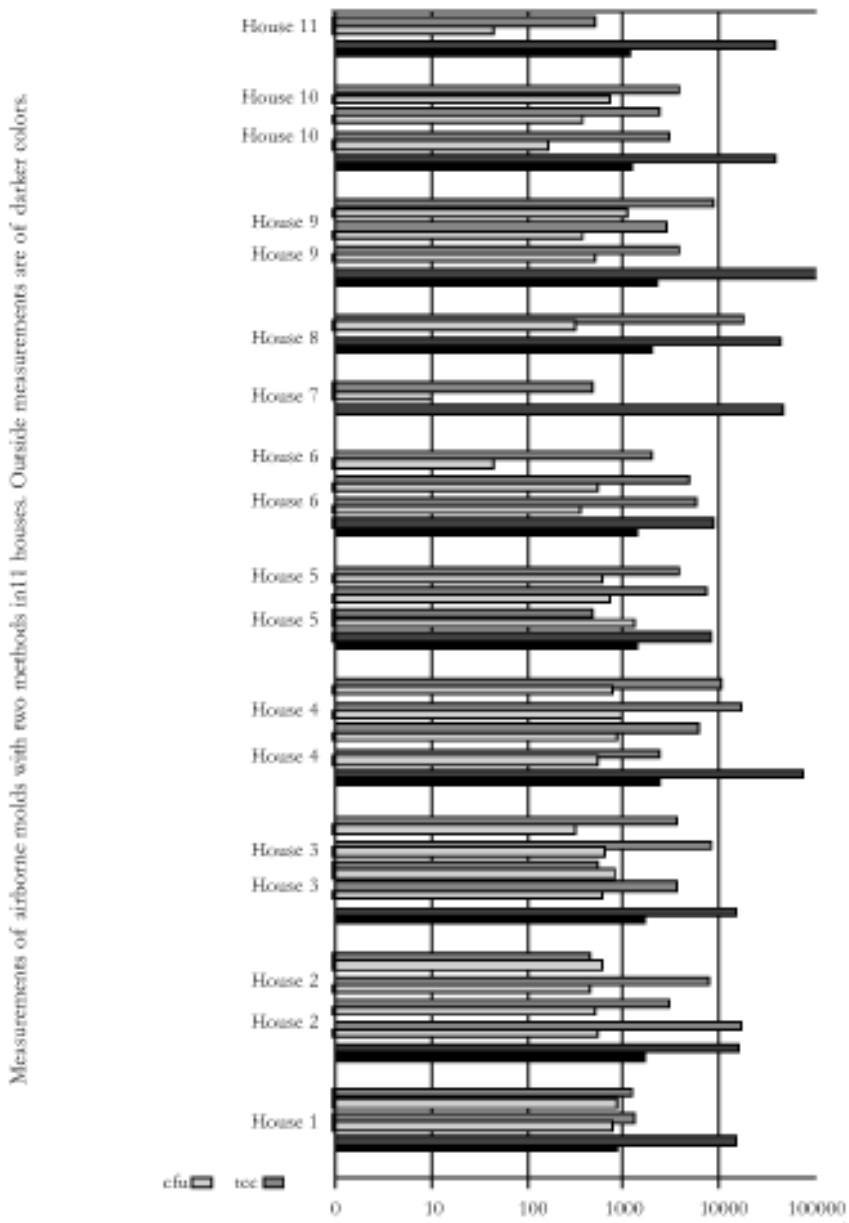
The variation is irregular. The results show that the cfu- and the tcc-levels can be low, compared with the outside reference air sample, all though there is a high concentration of microorganism generated inside the building (fig. 2, fig. 5). Both fig. 1 and fig. 2 show the expected results with lower numbers of cfu compared with tcc. These results would not confirm any indoor source of excessive mold contamination.

In fig. 2 the results of cfu and tcc at first sight looks as if they are not so far apart but that is caused by the logarithmic graph. Without this way to describe the results the column of the cfu values would be too small to make a comparison visible.

The results of the outdoor samples are always higher as the indoor samples. On the basis of this information from these samples, no estimation of expected health hazard can be determined.

It is important to point out that the microbial contamination of indoor air depends on a transport of molds from outside of the building to within or growth on surfaces inside of the building. The air itself is not a medium supporting microbial growth.

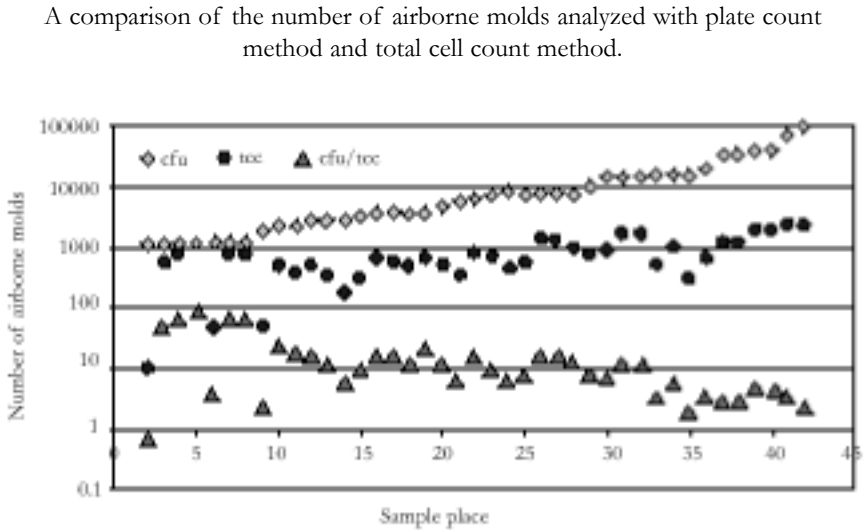
Fig. 2. Measurements of airborne molds outside compared to indoor air inside 11 houses.



The graph in fig. 3 shows not only the differences between cfu and tcc, but also the comparison cfu/tcc, which can be used as an age indicator of the source of the airborne contamination with molds. The higher values of cfu/tcc on the left side of the graph confirm fresher sources of the airborne molds and lower values on the right hand side older sources. Additionally, fig. 3 shows that in an older micro flora, many of the older cfu's of molds have not survived and can because of this not colonize the microbial plates in the lab.

These findings indicate that the results may depend on the time schedule of the release of mold spores and mycelia. The older the mold growth on a surface is, the more spores and mycelia parts are emitted to the air. This agrees with our own experiences, that new and visible mold growth may emit low numbers of airborne spores.

Fig. 3. The graph shows the differences between cfu and tcc but also the comparison cfu/tcc.



In fig. 4 the surface contamination (mold spores and mycelia/cm²) of the walls are compared to the resulting airborne molds. In some samples the airborne levels of molds are much higher then expected and in some samples much lower, but the visible resemblance between the patterns in the graph is intriguing.

Fig. 4. A comparison of molds from wall surfaces in the sampled room and the airborne molds in the air samples.

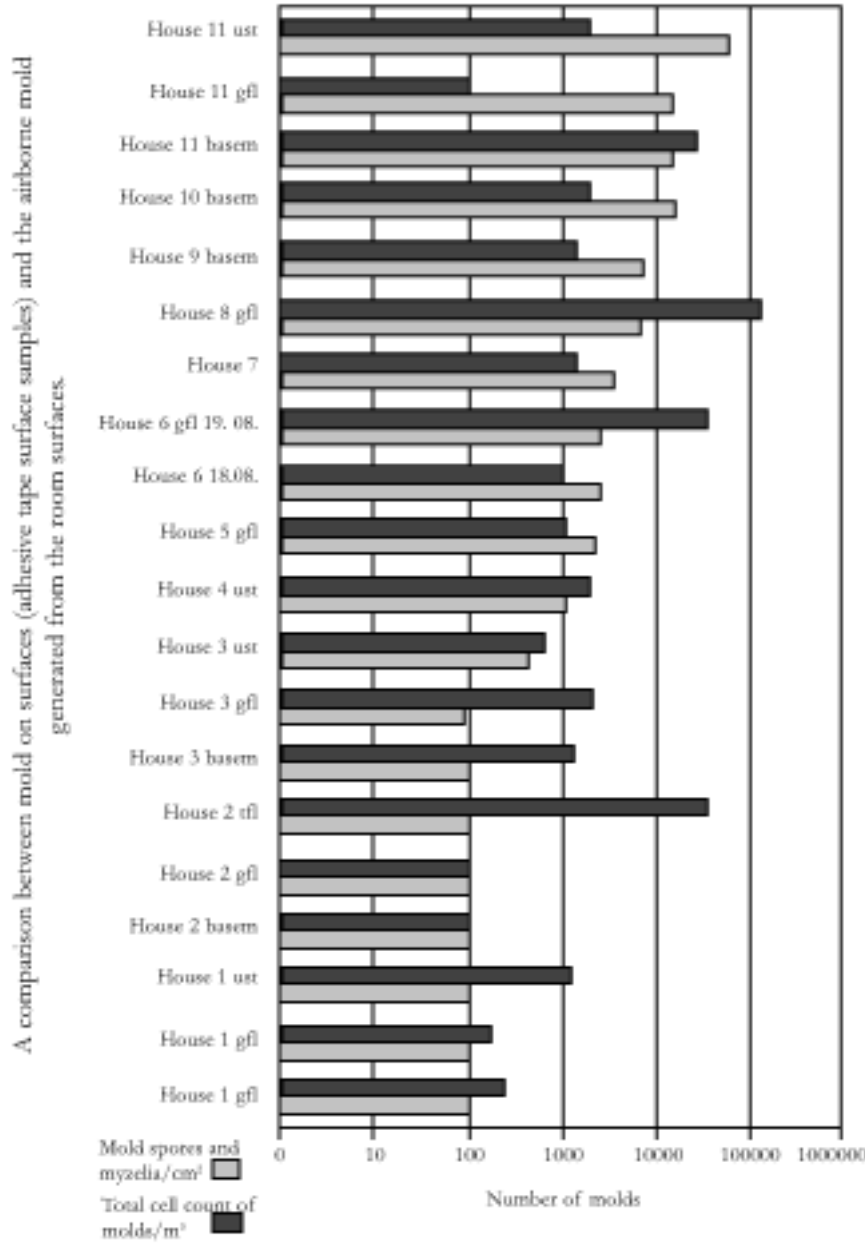
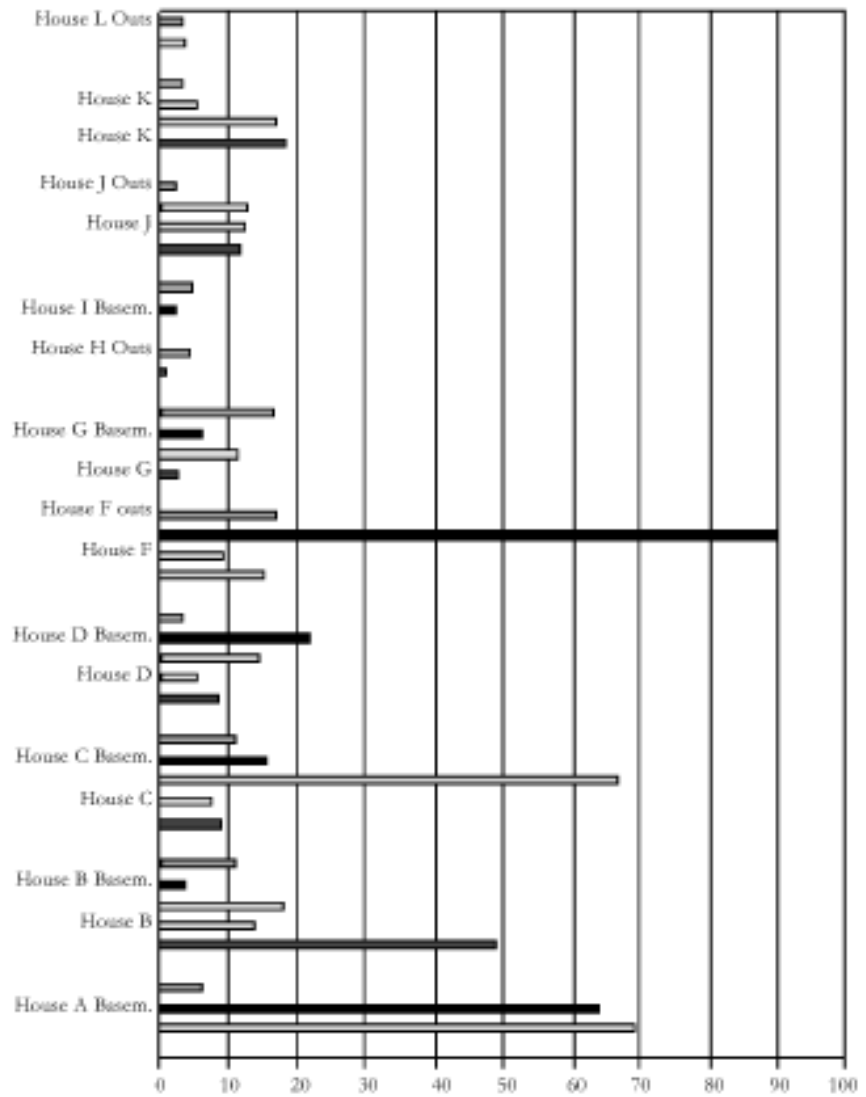


Fig. 5. In this graph the columns show the difference in age (cfu/tcc) between outdoor samples (dark grey) and indoor samples from different parts of the buildings.

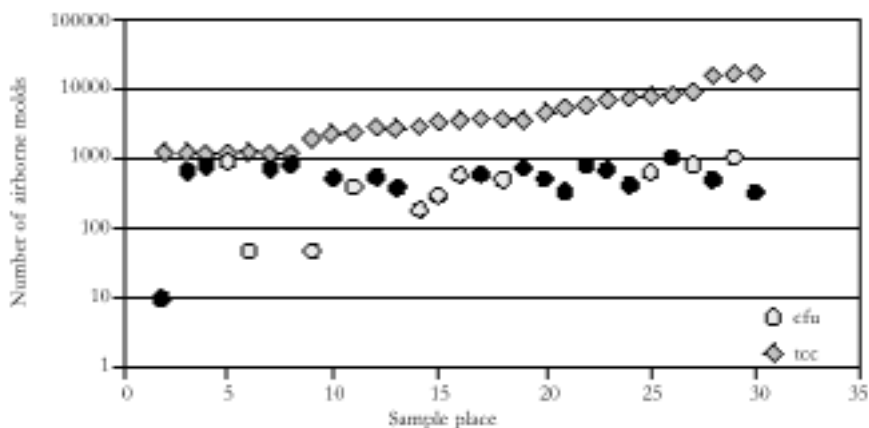


Through an air flow close to the contaminated surface of the building material the mold spores and fragments will get airborne and these microorganisms will show the same relation of cfu in percentage of the total cell count as those on the building material. The airborne molds can nevertheless naturally still have lower percentage due to them drying out in the dust before they get airborne a second time.

In fig. 5 the columns of outdoor samples (dark grey) and indoor samples are rarely of the same dignity and this indicate that the indoor and outdoor sources of airborne molds has come from different places. This information stresses the implications of the usefulness of relating indoor samples to not interfering outdoor samples.

In fig. 6. the importance of applying a reliable method of determining airborne mold becomes clear and the insufficiency of cfu analysis as sole information on airborne mold contamination in buildings is evident. Out of 29 samples with no indicative information, there were 18 indicative samples for indoor mold sources, when different methods of confirmation were used (cfu/tcc-evaluation).

Fig. 6. A comparison of the number of airborne molds analyzed with plate count method and total cell count method. Black dots are cfu values that fail to indicate an indoor mold source. This graph shows the unsuitability of using cfu levels as sole source of indication of airborne mold investigations.



Possible health effects of airborne molds are infections, toxicity and allergies, of which only infections are dependent on the viability of organisms. The threat of molds invading a human body, like for example the more hazardous pathogenic microorganisms, are very faint. The harmfulness of molds depends on their toxic or allergenic potential and the bodily response to their presence. The toxic and allergenic potential of airborne molds is dependent on the amount of biomass/m³ or

on the total number of microorganisms/m³. This toxic potential is however totally independent from the number of colony forming units/m³ (cfu/m³).

As a conclusion of all the results from this investigation, one can clearly distinguish that the attempt to correlate the number of airborne colony forming units to any types of health problems is unsuccessful. Even in the agricultural sector, no investigation has shown any correlation between the viability of microorganisms and the potential for inducing health problems. The correlation is just proven between total cell count/m³ and allergic alveolitis (Palmgren et al., 1986).

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