Analyse av muggsopp på overflater i porøse materialer og i luft med Mycometer-metoden

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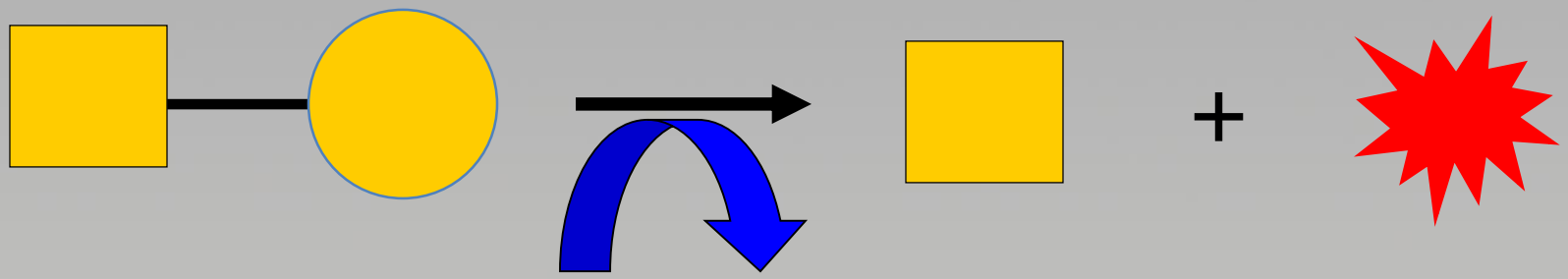
The Technology

Enzyme substrate

Fluorescence



β-**N**-**a**cetyl**h**exos**a**minidase (NAHA)

Dette enzymet er tilstede i alle muggsopp partikler: sporer, hyfer, hyfefragmenter og mikrofragmenter.

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NAHA facts

- Tilstede i både sporer og hyfer i alle filamentøse sopper
- Enzym aktiviteten er proportional med mengden av den totale sopp biomassen i prøven.
- Korrelerer med andre sopp parametre som ergosterol i komplekse prøver som jord og luft
- Er også funnet i ikke levende sopp partikler som mikrofragmenter

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Mycometer®-test

Mycometer-testen brukes for hurtigt å måle muggsopp on-site:

- på overflater
- inne i porøse materialer
- i luft

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Mycometer®-test

- Mest brukte metode til måling av muggsopp i Danmark.
- Hurtigmetode, prøvetaking og analyse kan utføres på stedet på mindre enn 1 time.
- Fortolkningskategorier (de samme for alle)
- Publisert i over 20 peer reviewed artikler.
- Verifisert av US-EPA i 2011
- Brukes av flere akkrediterte laboratorier.
- Dansk inneklima standard 3033

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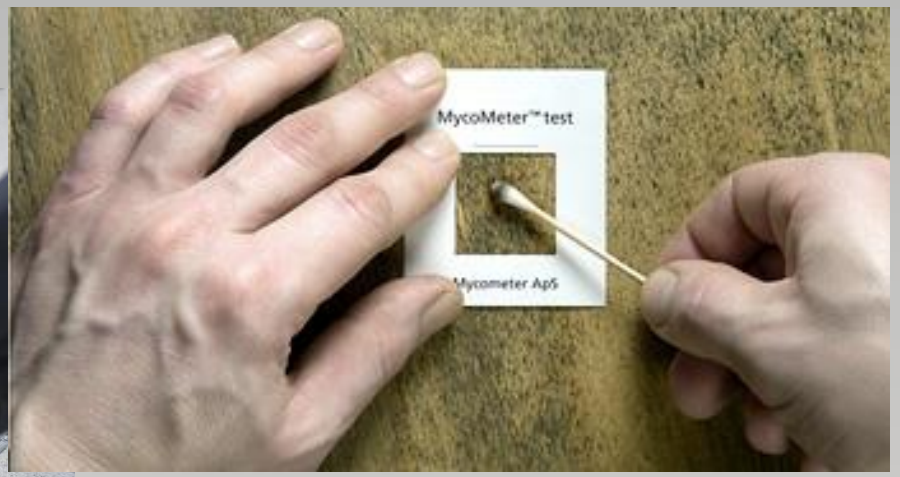
Mycometer-surface

Måling av muggsopp på overflater.



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Muggsopp inne i porøse materialer

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Måling av muggsopp i luft

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I USA har luftmålinger i lang tid vært miskreditert.

- Høy variasjon i målinger over tid.
- Falske negative resultater er vanlig
- Vanskelig tolkning av resultater.

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Typisk tidsvariasjon i den målte sporekonsentrasjonen (Distribution of Spores In a Colorado Home)

Time of Sample	Spore count
08:00	213
09:30	1,195
11:00	393
12:30	567
14:00	900
15:30	3,257

Source: Forensic-applications.com/moulds/sampling.html

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Hvorfor denne variasjon?

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Sopper i luften er partikler, de spres ikke ved diffusion.

Partikel størrelse (aerodynamisk diameter) µm	Tid for 1 m fald i stille luft (sek.)
1	8 timer
3	1 time
10	5,5 minutter

Unit density particles

Stachybotrys chartarum aerosols are gone in about 10 minutes in stagnant air

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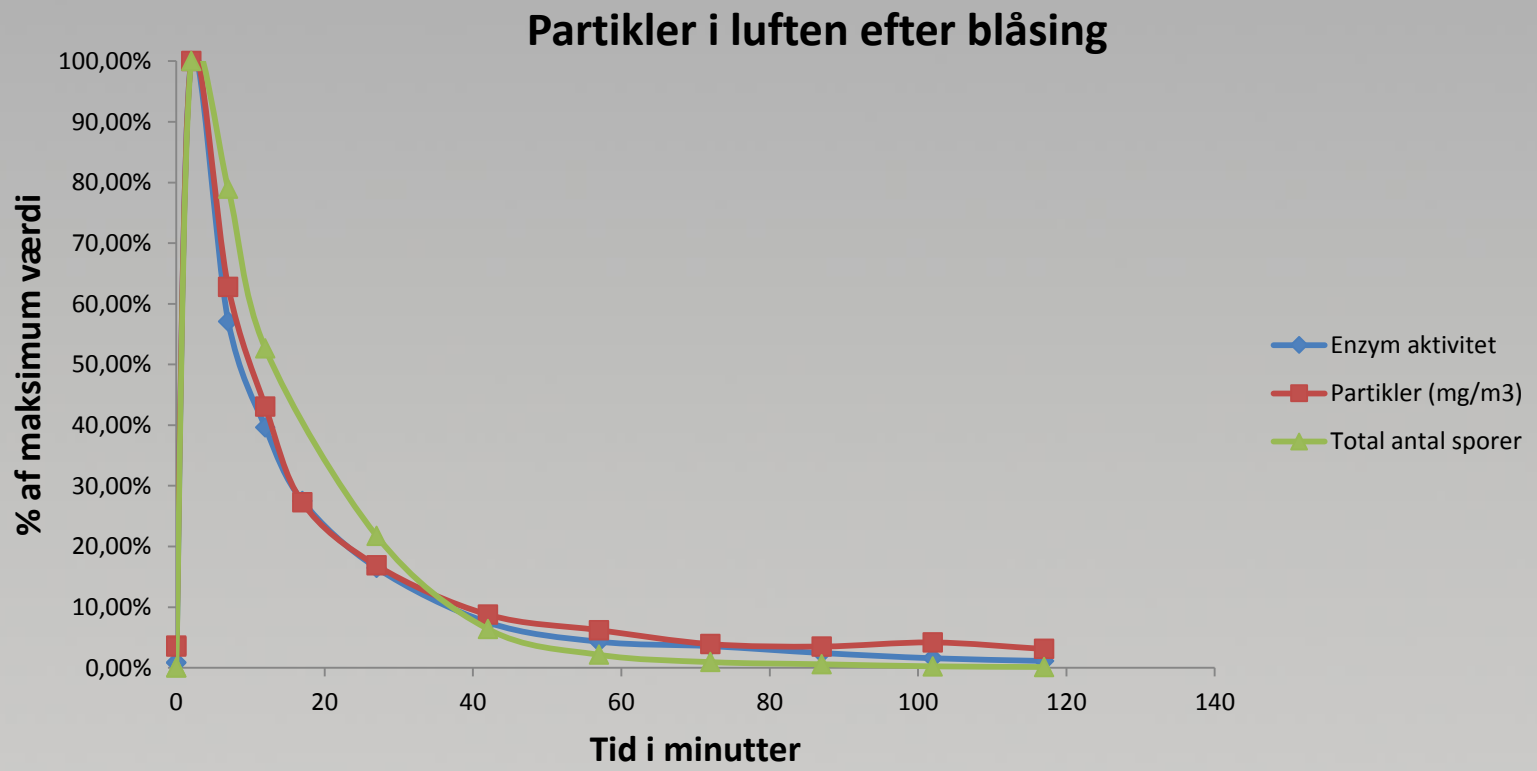
Partikler i luften etter blåsning



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Konsentrasjonen av muggsopp i luften er bestemt av aktiviteten i rommet det måles i.

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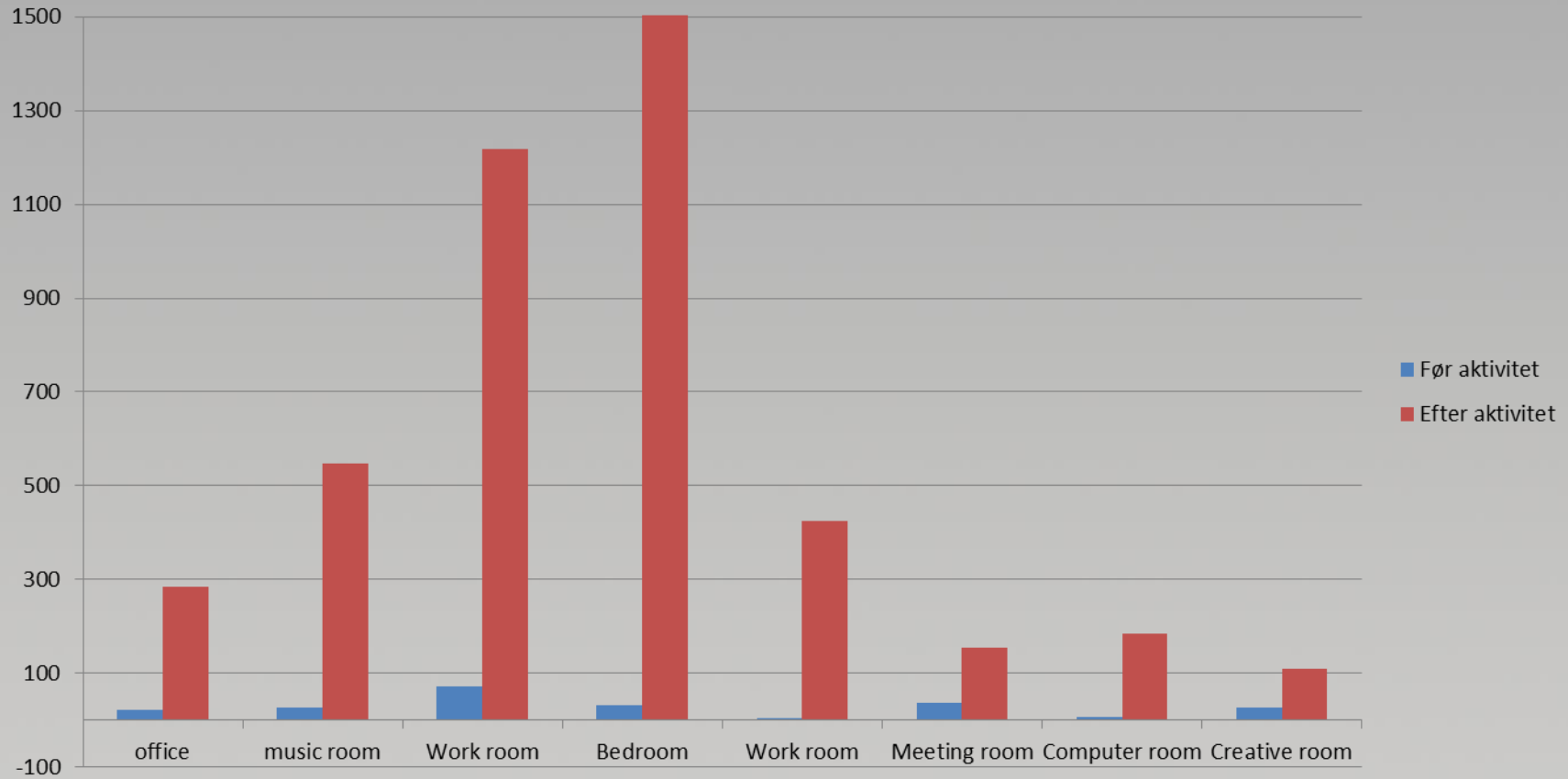
Passiv prøvetaking – før og etter aktivitet i rommene



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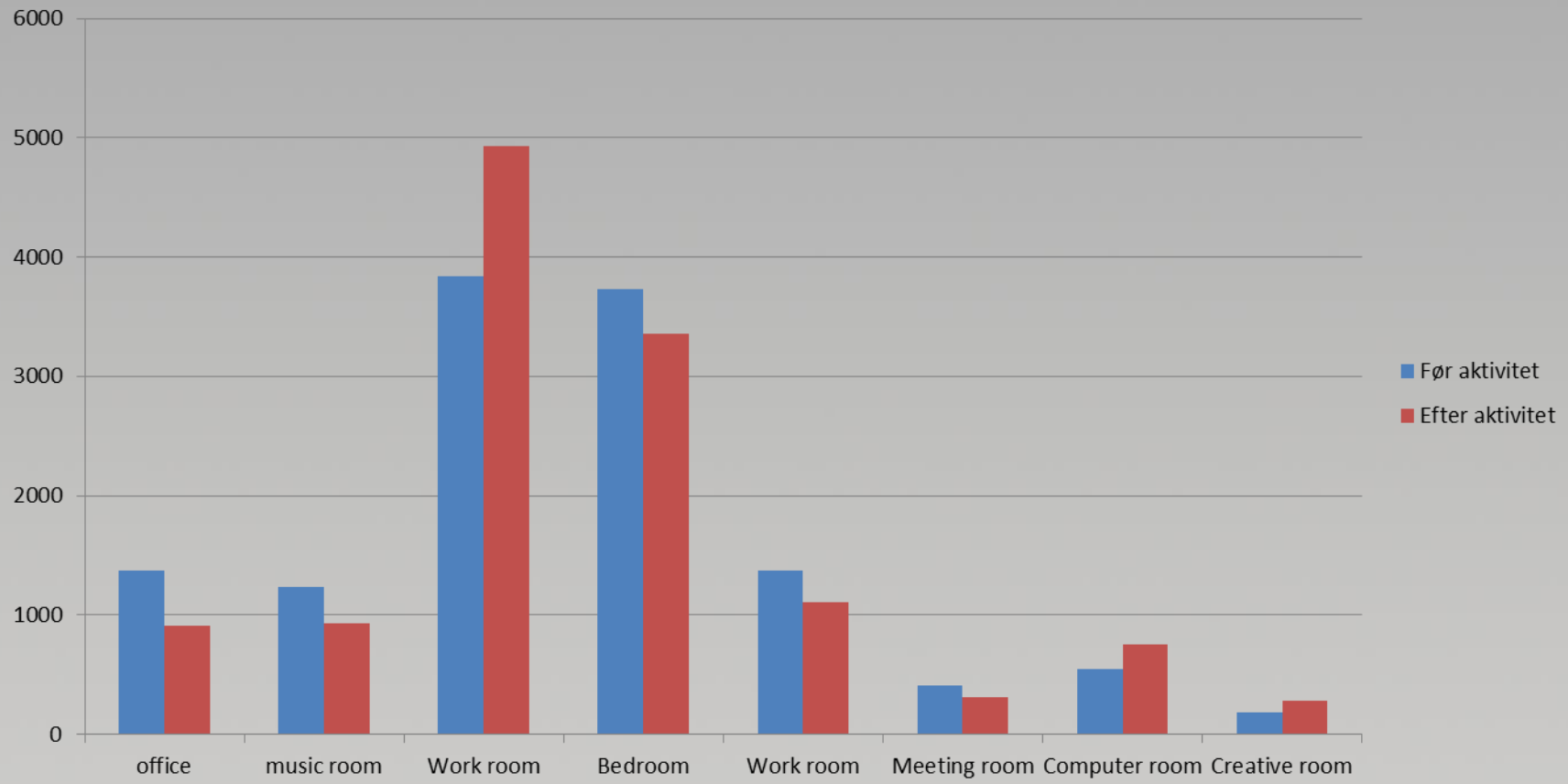
Aggressiv prøvetaking – før og etter aktivitet



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Aktiv/aggressiv prøvetaking

Aktiv eller aggressiv prøvetaking er tidligere blitt foreslått (Rylander, 1999).

Aggressiv prøvetaking brukes ved kvalitetskontroll etter asbest sanering (EPA guidance for clearing for reoccupancy after asbestos decontamination) og ved prøvetaking for Anthrax (McDermott, 2004).

Building: 106 Strandvejen, Roskilde

Sampling date: 08/02-2010

Sampled by: Jan C. Nielsen

Sampling volume: 300 L

Remarks: Both passive and aggressive sampling

Case #: JCN50377

		per m			
1	Basement, passive sampling	206	X		
2	1.st floor, passive sampling	450		X	
3	2. floor, passive sampling	690			X
4	Basement, aggressive sampling	12237			X
5	1.st. floor, aggressive sampling	2493			X
6	2. floor aggressive sampling	1727			X

Passive sampling
A = **MM-air value ≤ 350**
B = **350 < MM-air number ≤ 450**.
C = **MM-air value> 450**

Aggressive sampling
A = **MM-air value ≤ 900**
B = **900 < MM-air number ≤ 1700**.
C = **MM-air value> 1700**

Category A: Low content of mold in the air

Category B: Medium level of mold in the air.

Category C: High level of mold in the air.

Note: These Criteria are for non-mechanically ventilated buildings

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Konklusjon

Aktiv/aggressiv prøvetaking er helt avgjørende for å få representative prøver.

Jeg tror at det ved adaptering av aggressiv prøvetaking i vitenskapelige studier, vil være langt større sjanse for at man finner gode korrelasjoner mellom tilstedeværelse av muggsopp og helseplager.

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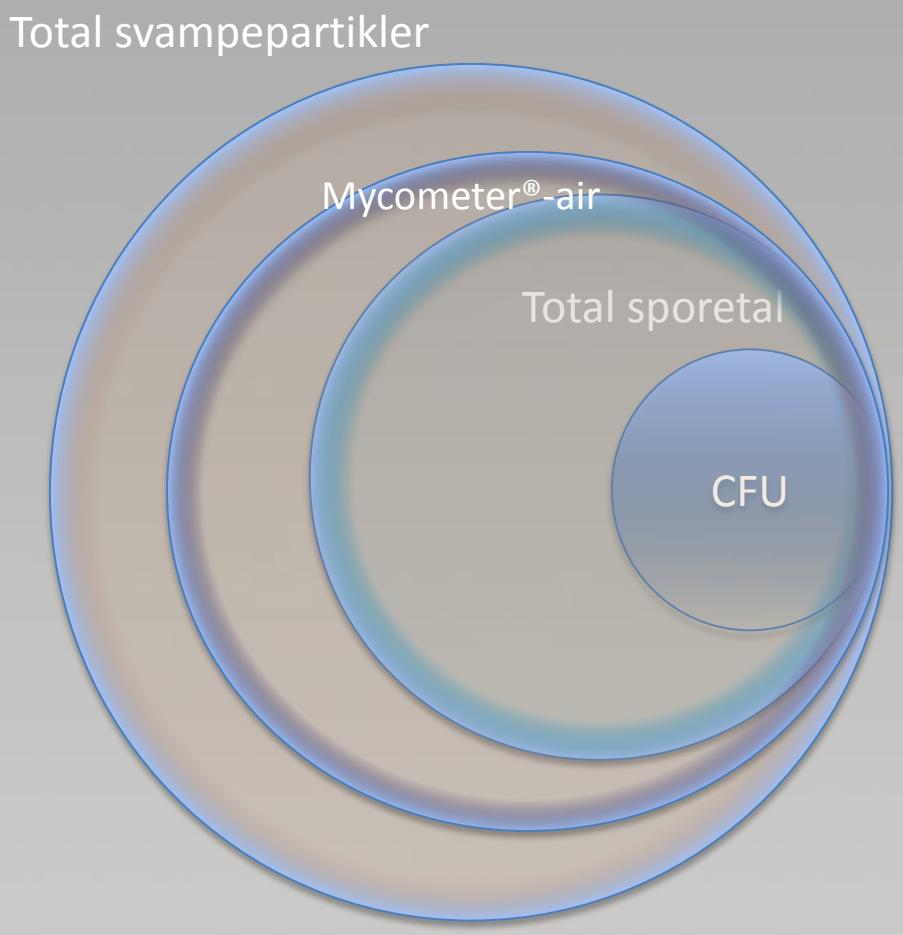
Hva måler forskjellige metoder?



CFU (KDE)= kolonidannende enheter, dvs. levende spiredyktige sporer bestemt på næringsagar. (Slit sampler + diverse) (1-10% af total sporer)

Total sporer = det totale antal igjenkjennelige sporer bestemt ved mikroskopering. Både levende og døde.
(Sporetraps, filter)

Mycometer®-air = levende + døde sporer, mikropartikler samt hyfefragmenter

Total sopp-partikler = levende + døde sporer, mikropartikler samt hyfefragmenter
(Ingen metode)

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Konklusion

Enzymmålinger (Mycometer-testen) er den målemetoden som måler på det største antall partikler sammenlignet med de metodene som er tilgjengelig i dag.

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Traditionelt har man anvendt utendørs prøver som referanse

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Sammenligning av innendørs konsentrasjon med utendørs konsentrasjon (samme Colorado Home sak)

Time	Indoor Spore Count	Outdoor Spore Count
10:00	971	6
13:15	16	112
15:23	33	102
18:06	426	133

Source: Forensic-applications.com/moulds/sampling.html

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Konklusjon

Utendørsmålinger kan ikke brukes som kvantitativ referanse for muggsopp innholdet i inneklimaet

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Tak for jeres opmærksomhed

For eventuelle spørgsmål: info@mycometer.com eller no@mycometer.com (Atle Sandven) eller i vores lille bod udenfor i pauserne

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Der er >6 ganger så mange sporer utendørs som innendørs (CFU)

En undersøkelse hvor 12.026 prøver ble analysert viste:

Median inne: 80 CFU/m^3

Median ute: 500 CFU/m^3

Kilde: Applied and Environmental Microbiology 2002, 68(4) 1743-1753

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How long does mold particles stay airborne?

- Theoretically, an average mold spore will fall by approx. 1 meter per hour in completely stagnant air
- It often goes a lot faster if e.g. the particles clump together or if the spores are large.
  - *Stachybotrys chartarum* aerosols are gone in about 10 minutes in stagnant air
  - *Penicillium* aerosols are gone in 20-30 minutes in stagnant air.

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TESTING THE HYPOTHESIS

Each result given is the mean of duplicate samples.

Type of room	7:30 am No activity prior to sampling	14:30 pm Activity prior to sampling	No activity/High activity (%)
Office, office building	22	284	8%
Music room, school	27	547	5 %
Work room, residential	73	1218	6 %
Bedroom, residential	32	2833	1 %
Work room, residential	4	425	1 %
Meeting room , office	37	154	24%
Computer room, school	8	184	4 %
Creative room, school	28	110	26 %
NAHA activity (FLU per m^3)		Mean	9 %

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Evaluation

- In all 8 cases the level of mould was much higher in the room after activity compared to no activity.
- It is a very small study but the results are very clear and backed up by causality

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Re-aerosolizing particles

- Walking/running
- Wind from an open window
- Vacuum cleaning
- Starting an HVAC system
- Starting a fan

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What has been suggested to overcome the variability?

- Sampling when there has been no activity for several hours.(it does gives much less variability, but does it give a true representation of what level of mould particles is actually present?)
- Long term sampling (it would even out much of the variability, but what if there are no activity? If it only give one mean values then it might not be so valuable)

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Alternative idea

- Creating a ”standardized activity level”

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Suggested protocol for agitated sampling

Blowing on surfaces two-three times with a handheld blower from approx. 2 meters distance. (Avoid dust reservoirs that are not normally stirred up).

Simulating a high but naturally occurring activity level.

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Testing the protocol for Agitated/Aggressive sampling

Each result given is the mean of duplicate samples.

Type of room	7:50 No activity prior to sampling	14:50 Activity prior to sampling	No activity as % of activity
Office, office building	913	1372	67%
Music room, school	929	1239	75%
Work room, residential	4927	3837	128%
Bedroom, residential	3360	3735	90%
Workroom, residential	1110	1377	80%
Meeting room , office	310	408	76%
Computer room, school	753	544	138%
Creative room, school	279	180	155%
NAHA activity (FLU per m^3)		Mean	103%

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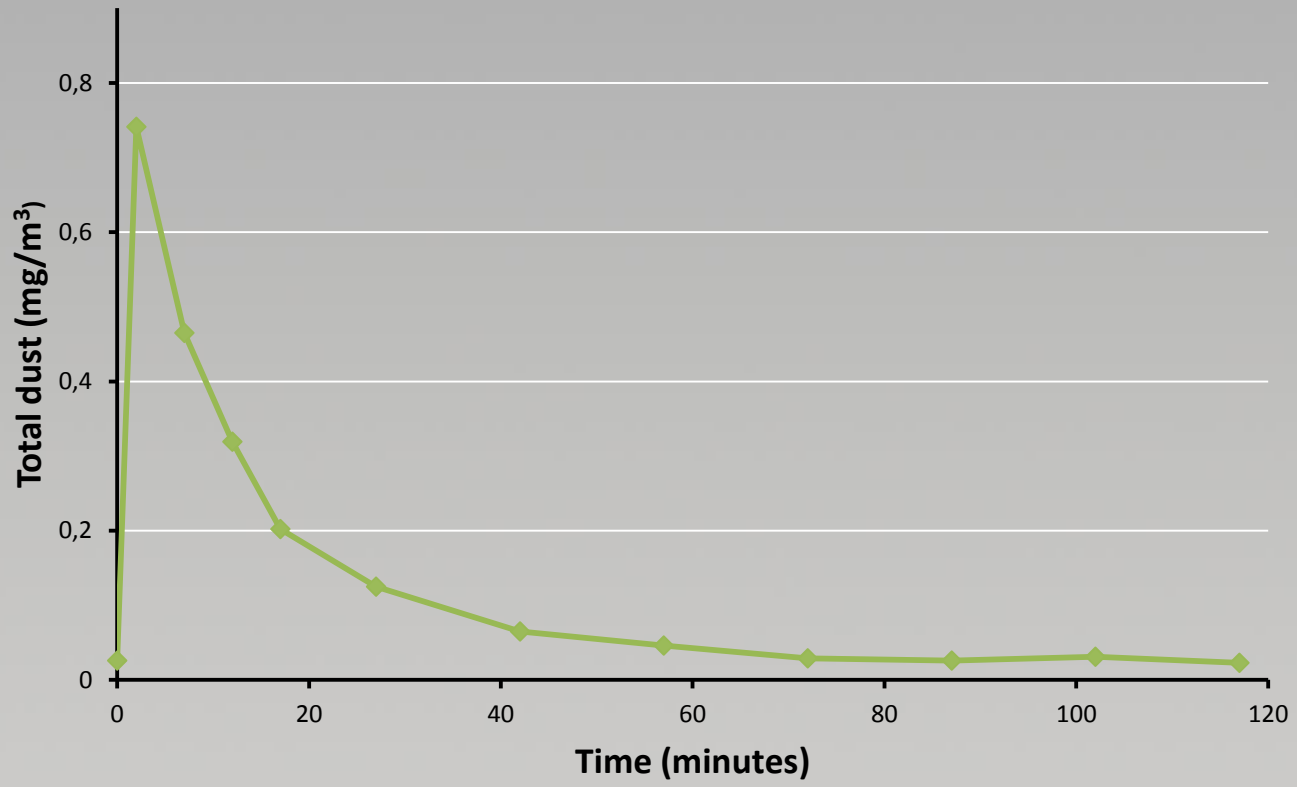
Total dust in air before and after aggressive sampling. Bedroom with non-visible mold growth.



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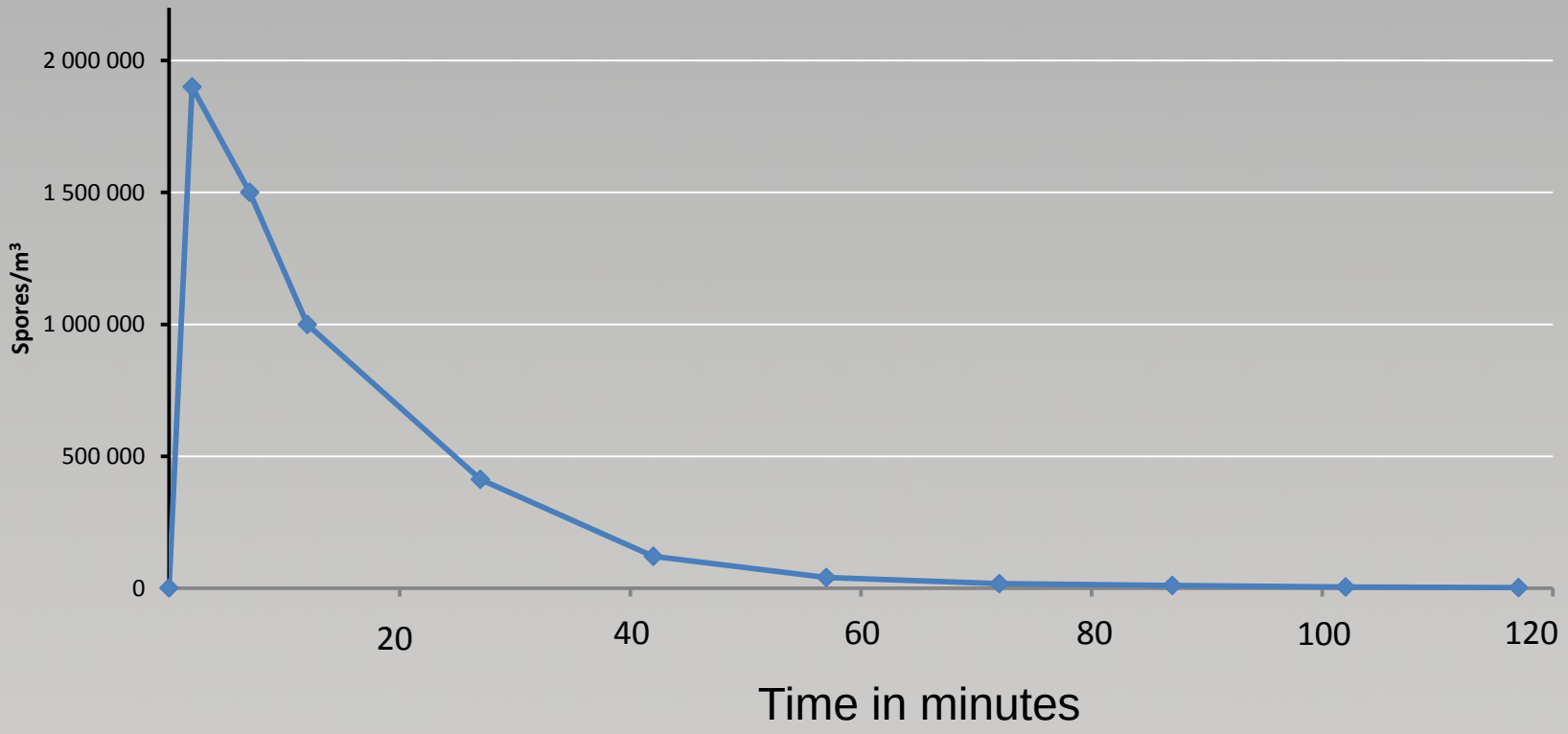
Total Spore count before and after aggressive sampling. Bedroom with non-visible mould growth



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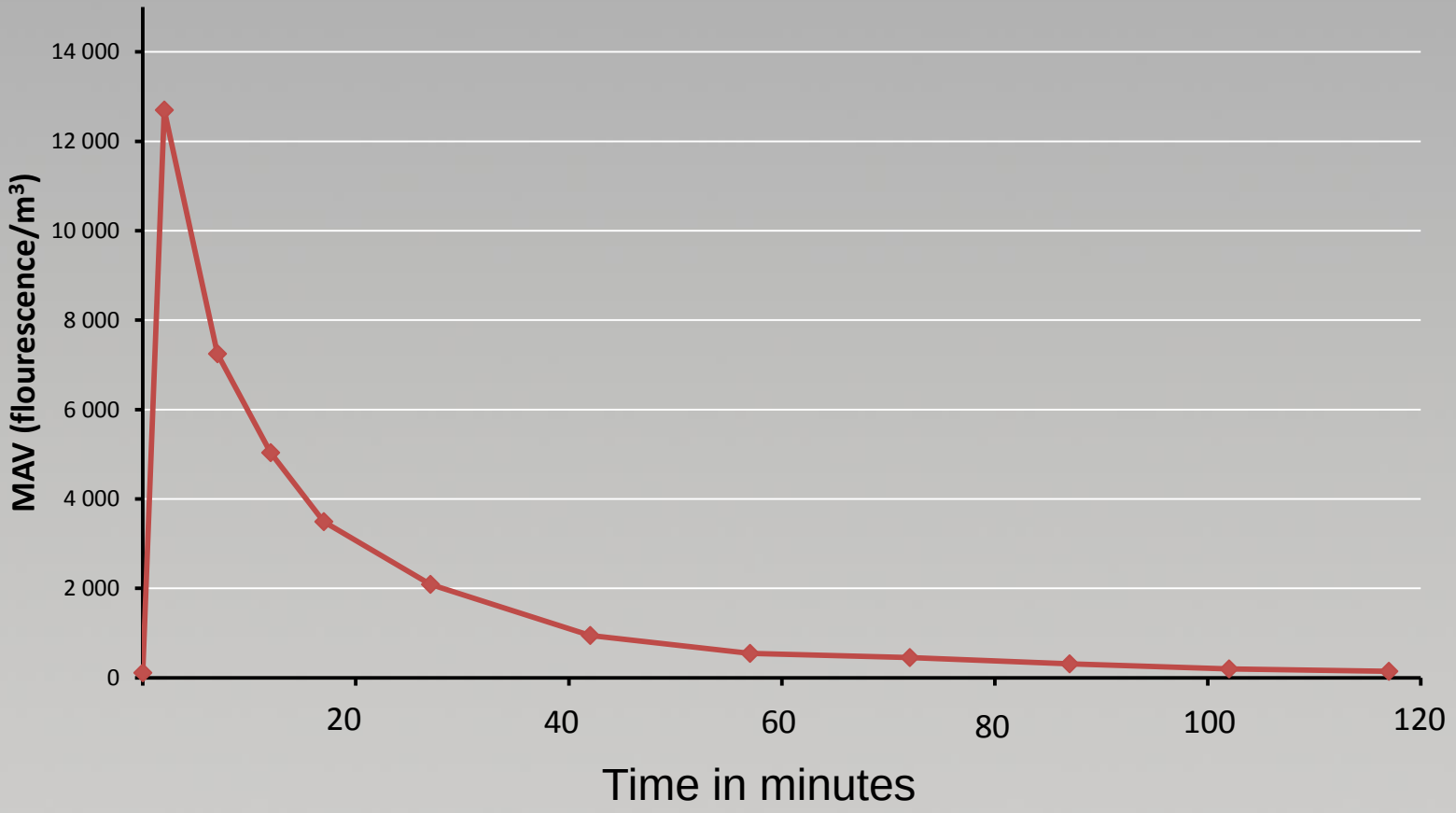
Enzyme activity in air before and after aggressive sampling. Bedroom with non-visible mould growth



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Thank you for your time

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Fungal propagules in air

- Viable culturable spores
- Viable non culturable spores
- Non-viable spores
- Hyphal fragments (> 1 µm, Viable or non-viable)
- Microfragments (≤ 1µm)

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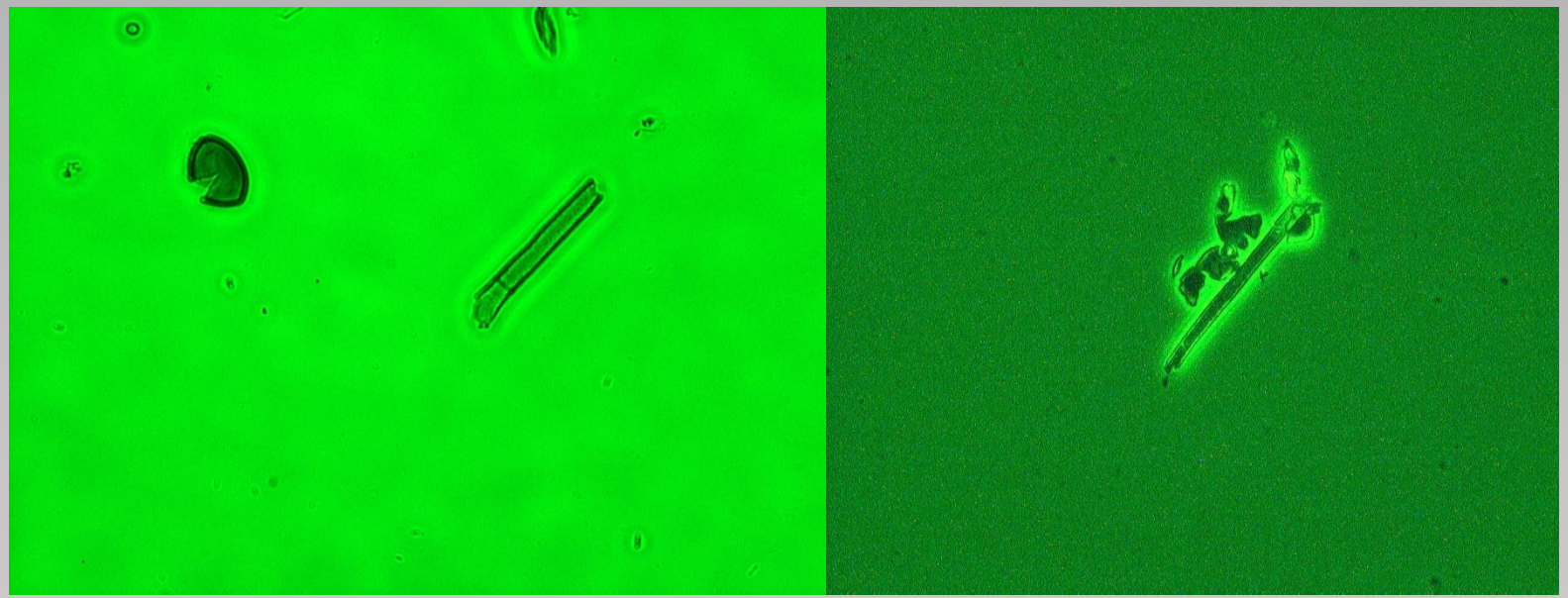
Formation of microfragments



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Aggressive air sampling protocol

Windows should have been closed at least 6 hours before sampling. Note if there is mechanical ventilation, dehumidifiers, air purifiers or the like, and if they are running.

1. Set up the pump and tripod.
2. Give the filter an ID and put it on the tube.
3. Set the timer or stopwatch to the desired time.
4. Put on the respiratory protection and set timer for 2 minutes.
5. Now *blow* **2-3 times on any surface from approx. 2 meter's distance** with the Makita blower. This should mimic high level of human activity e.g. vacuum cleaning or walking/running around or an open window on a windy day. Do not blow to release dust from reservoirs that are almost newer cleaned (e.g., between the lamella of a radiator).

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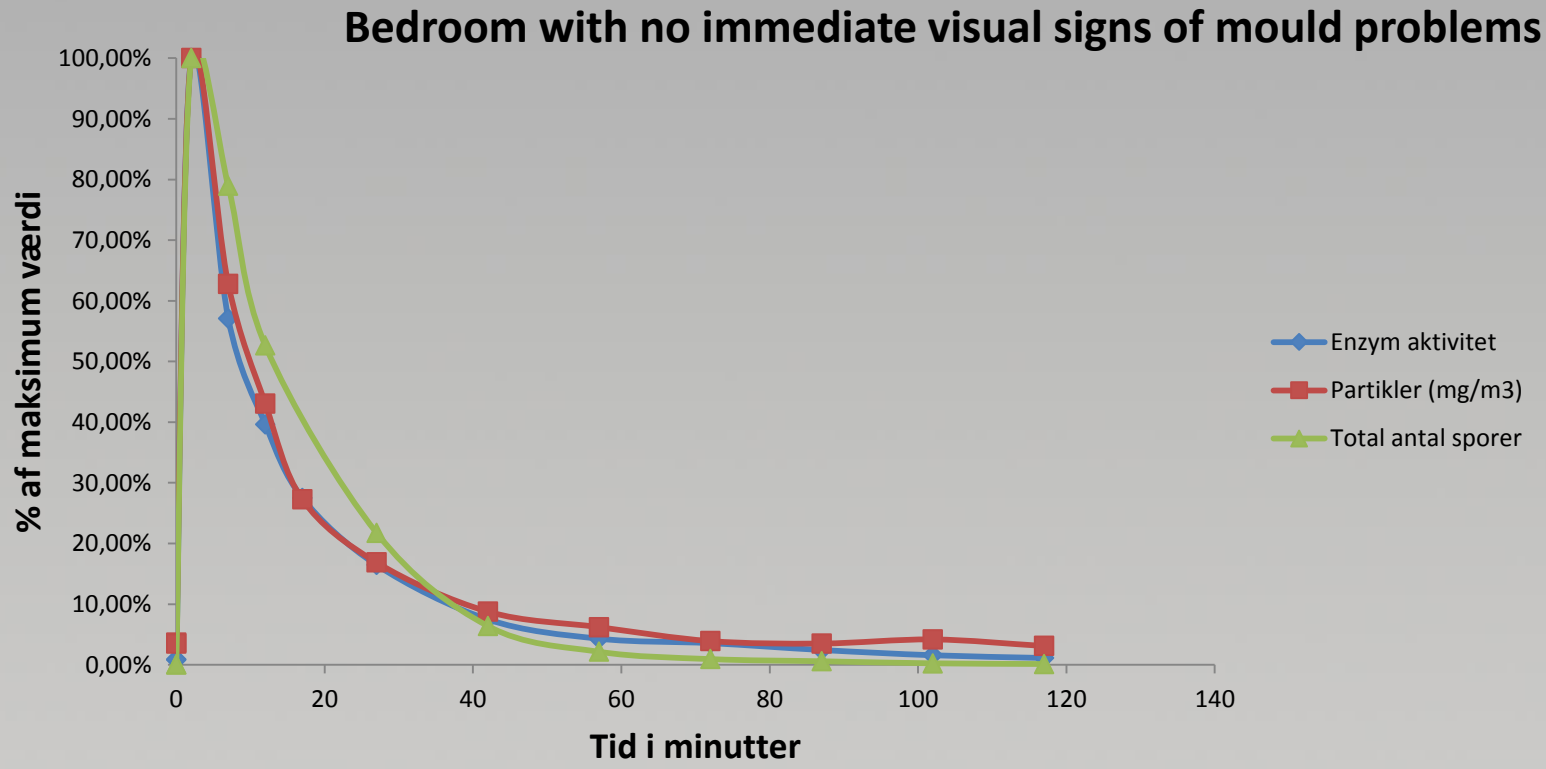
Settling of particles after aggressive sampling



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Results of the study

Table 5. The table shows minimum, maximum and median Mycometer-air Values of samples collected in both reference buildings and building with mold problems. Data from both passive and aggressive sampling is shown. n = 35.

Passive sampling			
	Reference buildings	Mold problem buildings (all rooms)	Mold problems buildings (only rooms with mold source)
Minimum – maximum	57-880	57–10723	167-10723
Median value (all rooms)	193	412	462
Aggressive sampling			
Minimum – maximum	113-2410	217-76233	707-76233
Median value	428	1468	4013

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A	MM-air numbers ≤ 900	Low level of mold in the air.
	The level of mold in the air is like that found in normal buildings with normal cleaning standards. Even with an A response and thus good air quality, it can not be excluded that there may be mold attack hidden in a building construction.	
B	900< MM-air numbers ≤ 1700	Medium level of mold in the air.
	The level of mold is higher than that found in normal buildings with normal cleaning standard. This may be due to accumulation of exogenous mold that accumulates due to poor cleaning standards. A category B result should always give rise to a more thorough inspection to look for a mold source.	
C	MM-air numbers > 1700	High level of mold in the air.
	The level of mold is significantly higher than that found in normal buildings. This may be because there is a source of mold in the room / building. A source may be growth of mold on / in buildings; but it can also be growth in firewood, potted plants, rotten fruit / vegetables, garbage, etc. Finally, a very poor cleaning standard could leave a large accumulation of external mold particles, that, for example by activity, can swirl up into the air.	

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A	MM-air numbers ≤ 350	Low level of mold in the air.
	The level of mold in the air is like that found in normal buildings with normal cleaning standards. Even with an A response and thus good air quality, it can not be excluded that there may be mold attack hidden in a building construction.	
B	350< MM-air numbers ≤ 450	Medium level of mold in the air.
	The level of mold is higher than that found in normal buildings with normal cleaning standard. This may be due to accumulation of exogenous mold that accumulates due to poor cleaning standards. A category B result should always give rise to a more thorough inspection to look for a mold source.	
C	MM-air numbers > 450	High level of mold in the air.
	The level of mold is significantly higher than that found in normal buildings. This may be because there is a source of mold in the room / building. A source may be growth of mold on / in buildings; but it can also be growth in firewood, potted plants, rotten fruit / vegetables, garbage, etc. Finally, a very poor cleaning standard could leave a large accumulation of external mold particles, that, for example by activity, can swirl up into the air.	

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Publications

- *The Use of Fluorogenic Substrates to Measure Fungal Presence and Activity in Soil.* Appl. *Environ. Microbiol. 64:613-617. M. Miller, A. Palojärvi, A. Rangger, M. Reeslev, A. Kjøller. 1998.*
- *Quantifying Mold Biomass on Gypsum Board: Comparison of Ergosterol and Beta-N-Acetylhexosaminidase as Mold Biomass Parameters*. Applied and Environmental Microbiology. *Vol. 69, No.7, p. 3996-3998. M.Reeslev, M.Miller, KF Nielsen. 2003.*
- *Analytical Instrument Performance Criteria: Application of a Fluorometric Method for the Detection of Mold in Indoor Environments*. *Applied Occupational and Environmental Hygiene. Vol. 18, No.7, p. 499-503. D Krause, YY Hamad, L Ball. 2003.*
- *The Mycometer™-Test: A New Rapid Method For Detection And Quantification Of Mold In Buildings.*
  Proceedings of Healthy Buildings 2000, Vol. 1, p.589-590. M.Reeslev and M. Miller. 2000.

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Publications

- *Nagase Activity In Airborne Biomass Dust And Relationship Between Nagase Concentrations And Fungal Spores*. Aerobiologia Vol. 19, 97 – 105. A.M., Madsen. 2003.
- *Application of a Fluorometric Method for the Detection of Mold in Indoor Environments*. (2003), D.Krause. Applied Occupational and Environmental Hygiene Volume 18(7): 1–5.
- *Successful Mold Growth Remediation in HVAC Systems*. P Buckmaster. Occupational Health and Safety, January 2008.
- *Airborne enzyme measurements to detect indoor mould exposure*. Journal of Environmental Monitoring, V.12, p.2161–2164. R. Rylander, et al. 2010
- *Fluorometric detection and estimation of fungal biomass on cultural heritage materials*. Journal of Microbiological Methods 80 (2010) 178–182, R Mitchell, et al (Harvard) 2010

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- *Beyond LEED, Pre and Post Occupancy Evaluations for New Buildings*. P Buckmaster. Synergist, May 2011.
- *Aggressive Sampling, Improving the Predictive Value of Air Sampling For Fungal Aerosols*. M. Reeslev, M. Miller, JC Nielsen, L Rogers. Proceedings of Indoor Air Conference, ISIAQC. June 2011, Austin Texas.
- Airborne enzyme measurements for the identification of mouldy buildings. Rylander R, Reeslev M, Hulander T. . J Environ Monit, 2010; 12:2161-2164
- Airborne enzyme in homes of patients with sarcoidosis. Terčelj M, Salobir B, Rylander R. Env Health 2011; 10; 8-13.
- Nocturnal asthma and domestic exposure to fungi. Terčelj M, Salobir B, Narancsik Z, Kriznar K, Grzetic-Romcevic T, Matos T, Rylander R. Indoor + Built Env 2012; submitted.