Fungal fragments: additional exposure burden in Norwegian sawmill industry

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Exposure to high levels of fungal spores has been associated with allergic alveolitis in workers from various occupational settings (Eduard 2006), including sawmills (Halpin, Graneek, Turner-Warwick, et al. 1994; Halpin, Graneek, Lacey, et al. 1994). Although a few studies have included quantification of fungal hyphal fragments in occupational exposure studies (Halstensen et al. 2007; Madsen et al. 2015) fungal exposure assessment has mostly been limited to spores or colony forming units. However, the fungal exposure may have been underestimated since *in vitro* aerosolization experiments of fungal cultures have shown release of fungal fragments of different sizes including large fragments (>1µm) and fragments in the submicrometer range (<1µm) in addition to spores (Górny et al. 2002; Madsen et al. 2005; Afanou et al. 2014). Exposure to fungal submicronic fragments has been suggested to worsen the health outcomes of fungi (Górny et al. 2002; Cho et al. 2005), but their discrimination in complex bioaerosols has not been possible until recently (Afanou et al. 2015). The aim of this study is to assess the personal exposure to fungal particles including submicronic fragments in Norwegian sawmills using immunodetection with field emission scanning electron microscopy (FESEM).

Full-shift personal samples of thoracic dust were collected using GK2.69 BGI cyclones at a flow of 1.6L/min in combination with standard aerosol cassettes loaded with polycarbonate filters (37mm, pore size 0.8µm). Collected particles were immobilized on the filter with poly-L-Lysine and vapor-fixed with glutaraldehyde before being subjected to immunolabeling as described by Afanou and coauthors (Afanou et al. 2015). Spores, larger and submicronic fragments were quantified using FESEM.

Preliminary results based on samples from four workers reveal the presence of fragments containing fungal antigens (Figure 1). The concentrations of larger fungal fragments were similar to that of fungal spores in the samples analyzed so far (Table 1 & 2). The percentage of submicronic fragments was below 1% (Table 2).

Table 1: Exposure of sawmill workers to fungal spores and fragments during one day shift

	Particle concentration (x 10^5 m^3)				
Worker number	Spores	Submicronic fragments (0.2-1 µm)	Larger fragments (>1 µm)	Total Fragments	Total Particles
1	3,7	0	3,7	3,7	7,4
2	10	0,2	6,6	6,8	17
3	0,7	0	0,5	0,5	1,2
4	0,7	0	0,8	0,8	1,5

Table 2: Distribution of fungal spores and fragments

	Percentage (%)		
Worker number	Spores	Submicronic fragments (0.2-1µm)	Larger fragments (>1 µm)
1	50	0	50
2	59	1	41
3	58	0	42
4	47	0	53

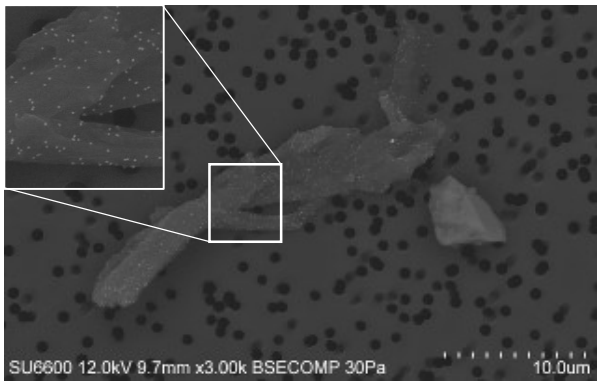

Figure 1: Micrographs of immunolabeled fungal fragments from air sampled at a Norwegian sawmill (white spots are the labelling nanogold particles)

To the best of our knowledge, this is the first study assessing the exposure levels of fungal fragments including submicronic fragments in an occupational setting. The results indicate that exposure to large fungal fragment particles (>1 µm) in sawmills is at a similar level as exposure to spores, and suggest that the total fungal exposure is twice as high as when quantifying spores only. Furthermore, hyphal fragments have been shown to have different toxicity than spores in *in vitro* studies. The fungal particle exposure of sawmill workers in different departments and seasons will be presented at the conference.

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