Assessment of Bioaerosols and Inhalable Dust Exposure in Swiss Sawmills

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An assessment of wood workers' exposure to airborne cultivable bacteria, fungi, inhalable endotoxins and inhalable organic dust was performed at 12 sawmills that process mainly coniferous wood species. In each plant, samples were collected at four or five different work sites (debarking, sawing, sorting, planing and sawing cockpit) and the efficiency of sampling devices (impinger or filter) for determining endotoxins levels was evaluated. Results show that fungi are present in very high concentrations (up to 35 000 CFU m$^{-3}$) in all sawmills. We also find that there are more bioaerosols at the sorting work site (mean $\pm$ SD: 7723 $\pm$ 9919 CFU m$^{-3}$ for total bacteria, 614 $\pm$ 902 CFU m$^{-3}$ for Gram-negative, 19 438 $\pm$ 14 246 CFU m$^{-3}$ for fungi, 7.0 $\pm$ 9.0 EU m$^{-3}$ for endotoxin and 2.9 $\pm$ 4.8 g m$^{-3}$ for dust) than at the sawing station (mean $\pm$ SD: 1938 $\pm$ 2478 CFU m$^{-3}$ for total bacteria, 141 $\pm$ 206 CFU m$^{-3}$ for Gram-negative, 12 207 $\pm$ 10 008 CFU m$^{-3}$ for fungi, 2.1 $\pm$ 1.9 EU m$^{-3}$ for endotoxin and 0.75 $\pm$ 0.49 mg m$^{-3}$ for dust). At the same time, the species composition and concentration of airborne Gram-negative bacteria were studied. *Penicillium* sp. were the predominant fungi, while *Bacillus* sp. and the Pseudomonadacea family were the predominant Gram-positive and Gram-negative bacteria encountered, respectively.

Keywords: airborne bacteria; airborne fungi; endotoxin; impinger; occupational health; wood workers

INTRODUCTION

In sawmills, workers handle many organic materials and are therefore exposed to high levels of organic dust (Alwis et al., 1999; Cormier et al., 2000; Demers et al., 2000). This could represent a risk of occupational diseases since dust from softwood (conifers) has been associated with a wide variety of upper and lower respiratory track effects as well as eye irritation and dermatitis (Enarson et al., 1990; Demers et al., 1997). Several investigations of wood workers have shown that certain work-related symptoms, particularly a decline of lung function, were more frequent among employees of sawmills than among control groups (Hedenstierna et al., 1986; Dahlqvist et al., 1992; Dahlqvist and Ulfvarson, 1994; Mandryk et al., 1999, 2000). Moreover, allergic alveolitis is known to be caused by airborne spores in Swedish sawmills (Wimander and Belin, 1980; Halpin et al., 1994). Before sawing, timber is always kept outdoors in damp conditions that favour mould and bacterial growth. Stored timber is often colonized by bacteria (Greaves, 1971; Rossell et al., 1973) and fungi (Levy, 1975) and therefore contains many non-infectious micro-organisms that can cause health problems. Wood dust and micro-organisms have both been suggested to induce occupational asthma and other respiratory symptoms, but few studies have assessed dust and microbial exposure levels in sawmills. Some studies in Canada, New Zealand, Australia and Norway (Eduard et al., 1993; Mandryk et al., 1999, 2000; Cormier et al., 2000; Douwes et al., 2000) have assessed one or more bioaerosols in sawmills. To date, no published studies have been carried out in Switzerland to analyse bioaerosols and inhalable dust in sawmills. According to the Swiss National Forest Inventory, conducted in 1993–1995 (Brassel and Brändli, 1999), forests in Switzerland are dominated by three species which account for 79.3% of the standing volume, i.e. 47.6% Norway spruce (*Picea abies*), 17.1% Beech (*Fagus sylvatica*) and 14.6% Silver fir (*Abies alba*). There are many forest communities throughout large areas of the French part of Switzerland that are dominated by

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All investigated sawmills process these wood species exclusively. The purpose of this work was to collect a comprehensive dataset for bioaerosol concentrations (endotoxins, bacteria and fungi) and organic dust in different sawmills and for different work sites (debarking, sawing, sorting, planing and sawing cockpit). As one sawmill study (Duchaine et al., 2001) has demonstrated a discrepancy in the results for endotoxin analyses depending on the sampling strategy (filter-sampling device versus impinger), we used both methods to compare the results. The composition of the community of Gram-negative bacteria was also investigated. This study was carried out as a part of a cross-sectional project to investigate occupational health in Swiss sawmills.

**MATERIALS AND METHODS**

**Sawmills**

Ten wood industries represented by 12 geographically separated sawmills located in the French part of Switzerland were chosen to represent various work conditions (small versus large). The number of employees varied from 4 to 160. The annual volume of processed wood varied between 1500 and 140 000 m³. Eight industries were small enterprises (<10 000 m³ annual processed wood) and only two industries were of medium size (>80 000 m³ annual processed wood). All sawmills processed coniferous wood species only (~90% Spruce, 10% Larch and/or Fir). Temperatures and relative air humidity were measured at each sampling site with an ECOLOG apparatus (Ecolog TH1, Elpro-Buchs). The mean summer temperature during sampling was 22 ± 1.0°C (17–28°C) and relative humidity was 55 ± 2.3% (45–66%).

All air samples were collected over a 3 month period during the dry part of summer. In two industries, samples were collected at 1 month intervals in two geographically separated buildings. These data from the same wood industry but from different plants were considered as independent variables in the statistical analyses. At each sawmill we collected samples at four or five different work sites, i.e. debarking, sawing, sorting, planing and in the sawing cockpit.

**Air sampling**

All micro-organism samples were taken in duplicate four times: twice in the morning and twice in the afternoon. For the filter-sampling devices, endotoxins and dust were sampled continuously for 4 h at stationary points. For the impinger devices, spot air samples were taken simultaneously within the filter-sampling period and at the same location. In each plant, we collected samples at four or five sites among the following: (i) in the cockpit which controls the saw, (ii) after the sawing point where the worker checks the process, (iii) at the sorting site, where the timbers are piled, (iv) at the planing site and (v) at the debarking site. All stationary samples were taken 1.5 m above the floor and near the worker to represent personal exposure during work.

**Bacteria and fungi**

Airborne bacteria and fungi were sampled with an impactor (MAS-100 Eco, MBV; Vevey, Switzerland) at a flow rate of 100 l min⁻¹. We sampled 20 l for fungi, 50 l for non-specific bacteria and 100 l for Gram-negative bacteria. Total cultivable bacteria were impacted onto Tryptone soja agar plates, Gram-negative bacteria onto MacConkey and fungi onto Dichloran glycerol plates (all from Oxoid, Basel, Switzerland). Fungi were incubated at 25°C for 5 days while bacteria were incubated at 30°C for 7 days, since at times we noticed new colonies after 6 days of incubation. All plates were checked daily for colony counts. Results are expressed in colony forming units (CFU) per cubic metre of air. The mean of the four samples from the morning and the four samples from the afternoon was used for the statistical analysis. The most frequent Gram-negative bacteria taxa were isolated and subsequently identified by using enzymatic test kits (API 20 E and API 20 NE, Biomérieux, France). Identification of the most frequent fungi genus was accomplished via macroscopic and microscopic examination, and reference to the manual *Medically Important Fungi—A Guide to Identification* (Larone, 1995).

**Dust sampling**

Inhalable dust was sampled on glass fibre filters at a flow rate of 2.0 l min⁻¹ using pocket pumps (MSA Escort Elf, Mine Safety Appliance Company, Pittsburgh, USA; or SKC pocket pump 210-1002, SKC Inc., USA) and IOM heads (SKC Inc.). Airflow was calibrated before and after field sampling with a piston calibrator (DryCal DC-Lite; Bios International, Pompton Plains, USA). One field blank was taken on each measurement day. The filters (taken out of the cassettes) were pre- and post-weighed on an analytical balance (Mettler, 0.001 mg sensitivity).

**Endotoxin sampling and analyses**

**Filter sampling.** Endotoxins were sampled onto polycarbonate filters (37 mm diameter, 0.4 µm pore size) placed in a ready-to-use polystyrene cassette (endofree cassette, Aerotech Laboratories, Inc., Phoenix, USA). Sampling was performed using a pocket pump (MSA Escort Elf, Mine Safety Appliance Company, or SKC pocket pump 210-1002, SKC Inc.) calibrated at 1.5 l min⁻¹. Airflow was calibrated before and after field sampling with a piston calibrator (DryCal DC-Lite, Bios International).
After sampling, cassettes were transported in a cold box to the laboratory within 3 h where they were stored at −20°C for 1–3 months to await endotoxin measurement. Endotoxins were extracted by shaking the filters at room temperature for 1 h in 10 ml of non-pyrogenic water in a 50 ml conical polypropylene tube. Filter extraction solutions were vortexed vigorously prior to drawing a sample for endotoxin analysis.

**Impinger sampling.** A maximum of four work sites in each sawmill were sampled with AGI-30 (Ace Glass Inc., Vineland, USA) and Gilian Aircon II pumps (Sensidyne, Clearwater, FL, USA) operating at a flow rate of 12.5 l min⁻¹ for 15 min. Sterile impingers were loaded with 20.0 ml of pyrogen-free saline (0.09% NaCl) prior to sampling and were kept on ice after sampling until they were returned within 3 h to the laboratory. Then the solutions were brought to 30 ml by addition of pyrogen-free saline. These solutions were stored at −20°C for 1–3 months to await endotoxin measurement. Prior to endotoxin assay, AGI solutions were thawed on ice and vortexed vigorously for 15 min.

**Endotoxin assay.** 0.1 ml of the AGI-30 or filter extraction solutions was analysed with a quantitative kinetic chromogenic Limulus amoebocyte lysate assay (Biowhittaker, Cambrex Bio Science, Verviers, Belgium) at 37°C with an automated microtitre plate reader. *Escherichia coli* O55:B5 endotoxin (Biowhittaker) was used as standard endotoxin. Results were expressed in units of endotoxin (EU) per cubic metre of air.

**Statistical methods**

The normality of the distribution was tested and log transformation or boxcox transformation or non-parametric tests were used if necessary. The effect of work sites and sawmills on bioaerosols and dust variables were tested using analysis of variance (ANOVA). The assumptions underlying the models were checked beforehand. Hypotheses of differences between afternoon and morning sampling were tested using Mann–Whitney *U*-test, and paired *t*-tests were used to test differences between samplers. Correlation between the different bioaerosols was tested using Pearson’s or Spearman’s rank correlation tests. All statistical analyses were performed using Systat software (SYSTAT Software Inc. products, Canada) or R software (free software). The data are generally presented as arithmetic mean values ± standard error (SE) or range (minimum and maximum values).

**RESULTS**

**Descriptive bioaerosol data**

**Fungi.** All investigated sawmills exceeded the Swiss occupational exposure guideline value of 1000 CFU m⁻³ of airborne fungi. On average, the concentration number of cultivable fungi per cubic metre of air was in the range 4318–35 130 CFU m⁻³ with a mean of 14 776 CFU m⁻³. We found no statistical difference between morning and afternoon sampling (Mann–Whitney test: *Z* = −1.55, *P* = 0.12, *n* = 46).

**Bacteria.** Two sawmills for total cultivable bacteria and one sawmill for Gram-negative bacteria exceeded the Swiss occupational exposure guideline values (10 000 and 1000 CFU m⁻³, respectively). The other sawmills were clearly within the Swiss occupational exposure guideline values. On average, for all investigated sawmills, the concentration numbers of cultivable bacteria and cultivable Gram-negative bacteria per cubic metre of air were, respectively, in the range 429–19 375 CFU m⁻³ with a mean of 3650 CFU m⁻³ and 20–1422 CFU m⁻³ with a mean of 285 CFU m⁻³. We found no statistical difference between morning and afternoon sampling (Mann–Whitney test: *Z* = −0.05, *P* = 0.9, *n* = 45 for total cultivable bacteria and *Z* = −0.7, *P* = 0.43, *n* = 46 for the Gram-negative bacteria).

**Endotoxins—Influence of sampling device.** For endotoxins, none of the sawmills exceeded the value of 100 or 50 EU m⁻³ recommended by some authors (Castellan et al., 1987; Heederick and Douwes, 1997; Rylander, 1997). On average, the concentration of endotoxin was in the range 0.4–17.8 EU m⁻³ with a mean of 3.9 EU m⁻³. When we compared the results obtained with the filter-sampling or the impinger device, we found that the impinger results gave significantly higher concentrations of endotoxins than the filter-sampling results (mean ± SE = 12.7 ± 14.4, versus 5.8 ± 7.3 EU m⁻³, air; paired *t*-test, *t* = −2.65, *P* = 0.013; *n* = 28). The two sampling strategies are marginally positively correlated (Spearman’s correlation, *r* = 0.36, *P* = 0.06).

**Dust.** On average, none of the sawmills exceeded the Swiss occupational exposure limit (OEL) of 5 mg m⁻³ for softwood. The concentration of dust was in the range 0.2–8.5 mg m⁻³ with a mean of 3.8 mg m⁻³. However, when we look separately at each measure, three workstations (always the sorting site) in three different sawmills greatly exceeded the OEL (up to 15 mg m⁻³ air).

**Correlation between bioaerosols.** A positive correlation exists between the level of endotoxins and the quantity of dust (Spearman’s correlation: *r* = 0.83, *P* = 0.0004). Total cultivable bacteria is also positively correlated to dust concentration (*r* = 0.58, *P* = 0.04). No other relationships between the different bioaerosols or between aerosol concentrations and temperature or relative humidity were found. The quantity of dust is also correlated to the size of the...
There are no significant differences among work sites for all bioaerosols and dust variables (Kruskal-Wallis one-way ANOVA, \( n = 8 \)). Mean and range (in parentheses) are shown.

### Influence of work site

As we found no differences in levels of bioaerosols between sawing, planing and debarking (\( P > 0.05 \), Table 1), these three variables were pooled in a new variable called ‘machines’ for statistical analyses. ANOVA on bioaerosols and dust concentration, using sawmill and work site as factors showed a strong significant effect of sawmill on all bioaerosols and dust measured (Table 2), while work site had a significant effect only on mean concentrations of airborne Gram-negative bacteria, endotoxins and wood dust (Table 2). Endotoxin and Gram-negative bacteria concentration are significantly greater at the sorting work site than at the machine work site (Fig. 1), while the concentration of wood dust is the lowest in the cockpit of the saw (Fig. 1). We observed that the other cultivable micro-organisms (total bacteria and fungi) show the same pattern as Gram-negative bacteria, i.e. the quantity of micro-organisms is the lowest at the ‘machine’ site and the highest at the sorting site. But these differences are not statistically significant (Table 2).

### Concentration and species of bacteria

In all sawmills, the nutrient plates for total cultivable bacteria were dominated by Bacillus spp. and the nutrient plates for cultivable fungi were dominated by Penicillium sp. and to a lesser extent by Aspergillus sp. The mean percentage of the total number of cultivable aerobic bacteria identified as Gram-negative (Gram-negative bacteria/total culturable bacteria) is 6.7 ± 0.6% (range: 0–29.6%). Among the Gram-negative bacteria, we have identified the genera Pseudomonas (Burkholderia cepacia 5x, Flavimonas oryzae/habitans 4x, Pseudomonas fluorescens 3x, P. putida 2x, P. aeruginosa 2x, Chryseomonas luteola 2x, Rahnsonia picketti 1x) as the predominant cultivable bacteria (number of colonies > 20% of total cultivable Gram-negative bacteria) at least once in each of the plants. In four plants we have detected Rahnella aquatilis and Pantoea spp. And finally, Ochrobactrum anthropi and Citrobacter freundii were found at least in one plant as a frequent cultivable bacteria compared with the other bacteria present on the nutrient agar (number of colonies > 10% of total cultivable Gram-negative bacteria).

### DISCUSSION

#### Dust

For wood dust, standards have been adopted in several countries (e.g. 5 mg m\(^{-3}\) for inhalable dust in the USA, Australia and Switzerland, 2 mg m\(^{-3}\) in the Netherlands, based on 8 h time weighted averages of inhalable dust, and only 2 mg m\(^{-3}\) of total dust in Sweden). In a recent literature review by Demers...
et al. (1997), a standard of 1 mg m\(^{-3}\) for softwoods was suggested to protect workers from non-malignant effects and there are also indications that wood dust levels \(\sim\)1 mg m\(^{-3}\) may cause reduced lung function (Eriksson and Liljelind, 2000). In the investigated sawmills, we have observed, on average, low concentrations of total wood dust; we have measured <1 mg m\(^{-3}\) of total dust in nine buildings of eight sawmills. Only three buildings in two different sawmills, the most productive ones (140 000 and 80 000 m\(^3\) of annual wood processed) and also the biggest in terms of the number of workers, exceeded, on average, 2 mg m\(^{-3}\), but were <5 mg m\(^{-3}\). When we examined each work site in these two plants separately, we found that the sorting sites always had the highest dust concentrations (up to 14.6 mg m\(^{-3}\)). In these work locations, steps to reduce exposure should be taken.

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\textit{Fungi}

All of the sawmills tested exceeded the Swiss occupational exposure guideline values, but it should be noted that there is no scientific basis for all Swiss guidelines concerning bioaerosols (Anonymous, 2003). Signs of restrictive lung impairment with an increase during a working week have been observed in workers exposed to mould spores with average values corresponding to 50 000 CFU m\(^{-3}\) air, but no impairment was established with daily average values corresponding to 2000 CFU m\(^{-3}\) air (Hedenstierna et al., 1986). In this study, all sawmills’ mean exposure to cultivable fungi and all measures carried out at different work sites were >2000 CFU m\(^{-3}\) air but none was >50 000 CFU m\(^{-3}\) air. Seven plants were >10 000 CFU m\(^{-3}\) and three work sites were >30 000 CFU m\(^{-3}\) air, indicating a high-grade exposure. Unfortunately, no dose–response relationship for

\begin{itemize}
  \item [Fig. 1.]{Mean amount + SE of different bioaerosols and dust per cubic metre of air sampled at different work sites (see details in text) for the 12 sawmills. Numbers beside the bars indicate the sample size.}
\end{itemize}
airborne fungi concentrations and decline of lung functions were available and it is impossible to clearly determine the influence of such high concentrations on workers’ health.

**Bacteria**

Only two plants exceeded the Swiss occupational exposure guideline values for total airborne bacteria and one plant was over the recommended threshold for airborne Gram-negative bacteria. However, we have to take into account that these assessment of bioaerosols were carried out with stationary samplers and for very short periods of time. Short-term samples are frequently more vulnerable to bias due to temporal variations in concentration than long-term samples. Consequently, they do not necessarily reflect the mean personal daily exposure of workers. The need to develop samplers that allow personal and long-term sampling of micro-organisms is crucial for the future.

**Endotoxin, influence of sampling device**

The levels of endotoxin measured were very low and do not seem to be an occupational risk in the sawmills investigated. This result contrasted with a study carried out in Canada with spruce/fir processing sawmills (Duchaine et al., 2000) and with a study from New Zealand (Douwes et al., 2000) where endotoxin exposure was high. Nevertheless, we have shown that the devices for sampling air for endotoxin analyses were important since results differed depending on the device used. Indeed, using impingers we measured significantly higher endotoxin levels than with filter-sampling devices. This might be due to the poor recovery efficiency of endotoxin from the filter during the extraction stage. This discrepancy of results between impingers and filters might be also due to the difference of sampling time between devices or due to the differences in particle size selection properties between samplers (see Duchaine et al., 2001). Nevertheless, this is consistent with another sawmill study that has shown that impinger sampling yielded endotoxin concentration estimates significantly higher than those obtained with filter-sampling measurements (Duchaine et al., 2001).

**Difference among work sites**

For all bioaerosols and dust measured, we observed a strong effect of the sawmill, i.e. exposure is very different from one site to another, while the work site had an effect only on mean exposure of airborne Gram-negative bacteria, endotoxin and wood dust. Concentrations of bioaerosols are greater at the sorting site than at the site where wood was debarked, sawed and planed. All planing machines were equipped with an air vacuum system and the sawing and debarking machines, which generate large quantity of wood dust, were situated away from the workers assigned to these work sites. Indeed, worker and stationary samplers were located at the end of the sawing or debarking timbers. This could explain the very low levels of wood dust measured at these sites. Another study in Canada has demonstrated the differences in exposure among work sites in sawmills (Duchaine et al., 2000). It was observed that dust concentrations were significantly higher at planing sites than at sorting sites and higher at sorting than debarking sites. For endotoxins, concentrations were significantly higher at debarking and sawing sites than at sorting sites. These differences of measured exposures could be due to different ways of working. More surprising is the relatively high level of bioaerosols encountered in the cockpit of the saw. One explanation might be that the worker was confined for a long time to a very small, isolated cabin without any ventilation system, which could favourable in maintaining a high level of micro-organisms.

**Species community**

Gram-positive bacteria, mainly *Bacillus* spp., largely dominated the non-selective nutrient plates. This result is in agreement with other studies (Rossell et al., 1973; Dutkiewicz, 1989). For fungal growth, agar plates were dominated by *Penicillium* sp. which is also consistent with other studies (Halpin et al., 1994; Cormier et al., 2000; Duchaine et al., 2000). Duchaine and co-workers (2000) have pointed out that the nature of contaminants found in eastern Canadian sawmills seems very different from those of studies performed in Europe, where *Rhizopus* sp. and *Paecilomyces* sp. dominated in sawmills that primarily processed Scots pine (Belin, 1980b; Dahlqvist et al., 1992; Eduard et al., 1992). In our case, we found large amounts of *Penicillium* sp. as in eastern Canadian sawmills (Duchaine et al., 2000) where spruce and fir account for the majority of timber processed as in Switzerland. Thus, growth of fungal species seems dependent on the species of wood. Gram-negative counts ranged from 0 to 29.6% (mean 6.7%) for viable counts. A broad spectrum of different species within the Pseudomonadaeae (ubiquitous bacteria) were predominant in all plants investigated. Within this family, the genera *B. cepacia* dominated. These bacteria are beneficial to the environment but can also cause human infections (Holmes et al., 1999). *R. aquatillis* and *Pantoea* sp. were found in four sawmills. *Rahnella* sp. belongs to the enterobacteriaceae, a group characterized by strong allergenic and immunotoxic properties. These bacteria occur often in wood processing activities in Poland (Dutkiewicz et al., 2001a; Krysinska-Traczyk et al., 2002). A recent study (Dutkiewicz et al., 2001b) has demonstrated that in Poland, the skin
response to the extract of *Rahnella* sp. was greater among workers processing coniferous wood (Scots pine *Pinus sylvestris*) than compared with workers processing deciduous wood. The presence of the genera *Pantoea* has already been reported on stored timber logs (Prazmo *et al.*, 2000). This species also has allergenic properties.

CONCLUSION

The Swiss sawmills investigated in this study contain a very high level of airborne fungi. The need to specifically evaluate the composition of the fungi community and its potential health effects on exposed workers seems to be crucial. Sorting sites were the most contaminated by dust, airborne Gram-negative bacteria and endotoxins. These data provide guidance about the locations and jobs in the mill where the exposures were the highest and could suggest effective control measures.

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REFERENCES