ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2012, Vol 19, No 2, 213-219
www.aaem.pl

Occupational exposure to airborne fungi in two Croatian sawmills and atopy in exposed workers

Maja Šegvić Klarić[1], Veda Marija Varnai[2], Anita Ljubičić Čalušić[2], Jelena Macan[2]

[1] *Department of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia*
[2] *Institute for Medical Research and Occupational Health, Zagreb, Croatia*

Šegvić Klarić M, Varnai VM, Ljubičić Čalušić A, Macan J. Occupational exposure to airborne fungi in two Croatian sawmills and atopy in exposed workers. Ann Agric Environ Med. 2012; 19(2): 213-219.

Abstract
Airborne fungi were collected over a one year period at 2-month intervals at 2 sawmills in Croatia (SM 1 and SM 2) processing mainly beech wood and oak wood. A questionnaire concerning respiratory symptoms and skin prick test (SPT) with common inhalatory allergens and moulds *Cladosporium herbarum*, *Alternaria alternata, Aspergillus niger, Penicillium notatum,* and *Rhizopus nigricans* were performed in 96 workers from the same sawmills. Average concentrations of airborne fungi were 1,696-7,316 cfu/m^3 in SM 1 and 1,706-4,819 cfu/m^3 in SM 2, respectively. Health hazardous levels of airborne fungi (above 10^4/m^3) were present only in SM 1. These levels were related to saw working sites and were season-dependent, i.e. present only during the summer. *Penicillium* (50-100%), *Paecilomyces* (43-100%) and *Chrysonilia* (33-100%) dominated among 17 fungal genera identified in both sawmills. Symptoms of rhinitis, asthma, and dry cough were most frequently recorded among analysed workers. SPT to moulds was negative in all tested workers, except one positive to *R. nigricans,* indicating that moderate airborne fungi levels found in the analysed sawmills were not related to IgE-mediated sensitization to moulds in exposed workers, even in atopics. Atopy was present among woodworkers in similar proportions to the general population of Croatia, suggesting that the wood-processing industry is not selective for atopic workers.

Keywords
airborne fungi, sawmills, *Penicillium*, *Paecilomyces, Chrysonilia*, skin prick test, atopy

INTRODUCTION

In the wood-processing industry, workers handling organic materials are exposed to various allergenic, toxic and carcinogenic substances originating from wood and micro-organisms present in wood dust [1, 2, 3]. The wood industry is recognized as an occupational environment with high levels of exposure to fungi [4]. Prior to processing, wood (timber) is kept outdoors, and exposed to variable environmental conditions which sometimes favour fungal growth [5]. During sawing, organic dust with fungal spores is dispersed into the air and workers could be continuously exposed to high levels of these airborne fungal particles. There are no internationally accepted occupational exposure limits for fungal spores or bacteria [6]. However, some countries have occupational exposure guidelines, e.g. in Switzerland, where the occupational limit for airborne fungi is 1,000 cfu/m^3 [5]. It has been suggested that an occupational concentration of airborne fungi > 10,000 cfu/m^3 should be considered a health hazard in non-sensitized subjects [4]. The wood-processing industry in Croatia exploits only a few tree species, including beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.), which account for up to 60% of the total wood supply. Fir (*Abies alba* Mill.) and spruce (*Picea abies* L.) share a lower percentage in the total wood supply (11.2%) [7]. To date, there have been no studies on occupational exposure to airborne fungi in the wood-processing industry in Croatia.

Address for correspondence: Maja Šegvić Klarić, Department of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Schrottova 39, HR-10000 Zagreb, Croatia. Tel./fax: +385 1 6394 493/+385 1 6394 494.
E-mail: msegvic@pharma.hr

Received: 27 July 2011; accepted: 29 December 2011

Fungal spores are known to cause several respiratory disorders based on different immunological and non-immunological pathways, including hypersensitivity pneumonitis, asthma, rhinitis, chronic bronchitis and organic dust toxic syndrome. Immunological responses are recognized as causes of hypersensitivity pneumonitis, and partly of asthma and rhinitis [4, 8]. Asthma and rhinitis can be phenotypes of atopy, disorder defined as a 'personal and/or familiar tendency, usually in childhood or adolescence, to become sensitized and producing IgE antibodies in response to ordinary exposures to allergens, usually proteins' [9]. However, IgE-mediated sensitization to fungi in occupational settings, including the wood-processing industry, to date has been rarely studied and with conflicting results. For example, high frequency of IgE-mediated sensitization to fungi was found in tobacco-processing workers and greenhouse flower growers [10, 11]. Additionally, the role of atopy as a risk factor for development of IgE-mediated sensitization to fungi in occupationally-exposed workers remains unclear [4, 12].

Therefore, the aim of this study was to evaluate levels of airborne fungi in two sawmills in Croatia and the atopy status in exposed workers.

MATERIALS AND METHODS

Study design. The study was designed as a longitudinal study regarding airborne fungi assessment in 2 Croatian sawmills (SM 1 and SM 2) situated near Zagreb, with follow-up every 2 months, from April 2008 – February 2009. Additionally, a cross-sectional study was conducted on wood-processing workers from the same sawmills regarding assessment of atopy. The study was performed in accordance with the Helsinki Declaration, and approved by the Ethical Committee of the Institute for Medical Research and Occupational Health in Zagreb.

Sampling and determination of airborne fungi. Airborne fungi were collected over a one year period (end of April 2008 – beginning of February 2009) at 2-month intervals at SM 1 and SM 2. Both sawmills have approximately 30,000 m^2 of production line, processing about 30,000 m^3 of wood annually. SM 1 processed solid beech wood (*Fagus sylvatica L.*), oak wood (*Quercus robur L.*), and fir wood (*Abies alba Mill.*), while SM 2 processed ash wood (*Fraxinus angustifolia Vahl.*), in addition to beech wood and oak wood. In each of the 2 sawmills, samples were collected in the morning during wood sawing operations at 10 indoor locations, including mechanical woodworking machines (5 sites), parquet production (3 sites) and wood drying (2 sites), as well as at 2 outdoor locations: sorting (2 sites) and wood storage (3 sites). Among these 15 sampling locations 7 were in the close proximity to the working saws (log band saws, cabinet band saws, circular saws, belt sanders), which have open suction systems. In addition, samples were collected at 2 outdoor locations which were at least 500 m distant from the sawmilling facilities. At all locations, no sampling was carried out on windy days. In each sawmill, as well as at the outdoor locations, samples were taken in duplicate on 2 consecutive days. Altogether, a total of 150 samples were taken in each sawmill (30 samples for a particular month), and 50 samples collected at the outdoor controls (10 samples for a particular month). Air temperature (°C) and relative humidity (%) were also measured by thermo-hygrometer (Boneco).

Fungi were sampled 1 m above the ground using an Mas 100 Eco air sampler (Merck) with 400 holes (hole to agar impactor) and Malt agar (BBL) plates supplemented with 50 mg/ml of streptomycin and 20,000 IU/ml of penicillin. The impaction velocity of the sampler is approximately 10.8 m/s and airflow rate 100 l/min. A volume of 50 l (30 s) was chosen for sampling. After field sampling, the plates were incubated at 25 ± 2°C, after which the developed fungal colonies were counted after 3, 5, and 7 days. The concentrations of airborne fungi were expressed as colony forming units per cubic meter (cfu/m^3). Fellers correction was not applied [13]. The airborne fungi were identified on the basis of their macro- and microscopic characteristics after subculturing on Czapek, Malt extract and Potato Dextrose agar, according to the manuals [14, 15].

Subjects and questionnaire. All workers exposed to wood dust at their work sites (sawing, cutting, debarking and planing of wood, and parquetry production) were invited to participate. Recruitment was on a voluntary basis after an invitation issued by their employers. All participants signed informed consent and were free to leave the study at any time. A total number of 96 wood-processing workers were examined, 35 from SM 1 and 61 from SM 2. Response rates were 98% for SM 1, and 84% for SM 2. An inclusion criterion for participating in the study was exposure to wood dust for ≥ 20 hours per week, and absence of contraindications for skin prick test. Most of the workers did not wear personal airway protection. The main characteristics of the study subjects (gender, age, duration of work exposure in the wood-processing industry, smoking habit) are presented in Table 3. Study protocol for the subjects included a medical interview according to a modified organic dust questionnaire, physical examination, and skin prick testing.

A questionnaire based on the 'Organic Dust Questionnaire' was completed by the physician who interviewed each subject [16]. Smoking habit was expressed as a dichotomous variable (smokers and non-smokers) and as a smoking index (number of cigarettes smoked per day x number of smoking years). Only current smokers were designated as smokers. The questionnaire recorded reports on the occurrence of work-related eye, respiratory, skin and general symptoms during the last 12 months. In this study, only upper and lower respiratory airway symptoms were analyzed. Upper respiratory symptoms included sneezing, rhinorrhea, nasal itching and nasal obstruction (not related to the common cold). The variable 'rhinitis' was defined with 2 or more upper respiratory symptoms being recorded. Lower respiratory symptoms included dry cough, phlegm (cough with expectoration), wheezing and dyspnea. The variable 'asthma' was defined as the recording of wheezing and/or dyspnea. Chronic bronchitis was defined as persistent cough and phlegm for more than 3 months per year, lasting for more than 2 years. Contraindications for skin prick test were evaluated for each subject during completion of the questionnaire and physical examination.

Skin prick testing. Skin prick testing (SPT) was performed in 94 subjects (test not valid in 2 subjects from SM 1) by a standard method [17] with a panel of common inhalatory allergens: grass pollen mixture, birch, hazel, weed (*Ambrosia elatior, Artemisia vulgaris*) pollens, mites *Dermatophagoides pteronyssinus, Dermatophagoides farinae,* and *Lepidoglyphus destructor,* cat and dog epithelia, and moulds *Cladosporium herbarum* and *Alternaria alternata* (Allergopharma, Reinbeck, Germany). Subjects were additionally tested with moulds *Aspergillus niger, Penicillium notatum,* and *Rhizopus nigricans* (Allergopharma, Reinbeck, Germany). SPT included testing with positive control solution (10 mg/ml of histamine hydrochloride) and negative control solution (buffer). Skin reaction (wheal) was evaluated after 15 minutes. The mean skin reaction (mean wheal diameter) was calculated according to the formula (D+d)/2, where D represents the largest longitudinal diameter and d its midpoint orthogonal diameter in mm. The difference between mean skin reaction to each allergen and negative control solution was used as a parameter of SPT reactivity. The results of SPT were considered positive when the mean wheal diameter was larger than the negative control by more than 3 mm. According to the definition by Johansson *et al.* [9], atopy was defined as positive SPT to at least 1 common inhalatory allergen and presence of rhinitis and/or asthma symptoms.

Statistics. The Kolmogorov-Smirnov test was used to verify whether the variables were normally distributed. Concentrations of airborne fungi were logarithmically transformed to normalise distribution. When normality was achieved, ANOVA and Bonferroni multiple comparison tests were used. Otherwise, Kruskal-Wallis test was applied, followed by Dunn's multiple comparison test. The Spearman's correlation coefficients between average fungal concentration at each location and ambient parameters (temperature and relative humidity) were also calculated.

Regarding the differences between workers from the 2 sawmills, categorical variables (gender, smoking status, positive SPT, atopy status) were analyzed by Pearson χ^2 test or Fisher's exact test if one or more expected frequencies were ≤ 5, normally distributed continuous variable (age) by Student's t-test, and non-normally distributed continuous variables (working exposure and smoking index) by Mann-Whitney U test. The tests were 2-tailed. Statistical analysis was performed using software Stata/SE 10.0 for Windows (StatCorp LP, TX, USA). The results were considered statistically significant at p value<0.05.

RESULTS

Levels of airborne fungi. Average concentrations of airborne fungi ranged between 1,696-7,316 cfu/m^3 in SM 1, and between 1,706-4,819 cfu/m^3 in SM 2 (Tab. 1). These average concentrations were significantly higher in April-May and July compared to other samplings. In SM 1, the level of airborne fungi was twice as high in April-May (6,215 cfu/m^3) and 1.5 times higher in July (7,316 cfu/m^3) than in SM 2 ($p<0.05$). In the subsequent periods of sampling, the concentrations were approximately 1,700 cfu/m^3 in both sawmills. The work site had a significant effect on the level of airborne fungi and influenced the data distribution. When the values obtained from samples taken near the sawmills (SM 1b, SM 2b) were separated from those taken at a distance (SM 1a, SM 2a), data were normally distributed in all 4 groups. Average levels of fungal particles were 3 (SM 2b) to 9 (SM 1b) times higher in the close proximity of the sawmills (Fig. 1) compared to samples taken at a distance ($p<0.001$). In July and September in SM 1, significantly higher levels of aeromycota were recorded in the proximity as well as at a distance (for July only) of the sawmills compared to SM 2 ($p<0.01$; $p<0.05$). Levels of >10,000 cfu/m^3 were recorded only in SM 1. Maximum concentration near the sawmills was measured in July (14,400 cfu/m^3). Also, in this period, fungal levels exceeded 1,000 cfu/m^3 in samples taken at few meters at a distance from sawing, while during other samplings, the levels of airborne fungi were between 500-1,000 cfu/m^3. Mean concentrations of fungal particles in outdoor air were significantly lower than in the sawmills ($p<0.001$), with the minimum detected in December (158 cfu/m^3) and maximum detected in July (710 cfu/m^3). A significant positive correlation was found between temperature and levels of airborne fungi in the sawmills and outdoor air (Tab. 1).

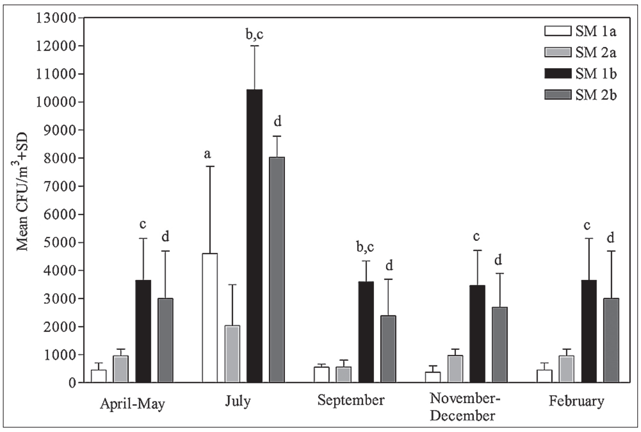

Figure 1. Concentration of airborne fungi at a distance (SM 1a and SM 2a), and in close proximity to sawmills (SM 1b and SM 2b).
[a] ($p<0.05$) SM 1a *vs* SM 2a;
[b] ($p<0.05$ or $p<0.001$) SM 1b *vs* SM 2b;
[c] ($p<0.001$) SM 1a *vs* SM 1b; [d] ($p<0.001$) SM 2a *vs* SM 2b.

Aeromycota frequency of isolation. Frequency of aeromycota in the 2 sawmills and outdoor air during the sampling period is presented in Table 2. Frequencies (%) of airborne fungi were calculated as positive samples in the total number of samples for each location. Airborne fungi ascribed to 17 genera were recovered from samples. Frequency of aeromycota in the 2 sawmills showed no particular difference. *Penicillium* was the dominant fungal genera which occurred in 50-100% of samples in both sawmills. This was followed by *Paecilomyces* spp. which was found in 43-100% of samples during the sampling period, except in February when it was found in lower frequencies (8% in SM 1 and 15% in SM 2). *Chrysonilia sitophila* showed a similar pattern, and was isolated from 33-96% of samples from April-May to November-December, and in February was recovered from only 8% of samples in both sawmills. Among other constantly present airborne fungi, *A. niger* peaked in April-May (71% and 65%), *Trichoderma* spp. (33% and 40%) and *Rhizopus* spp. (20%) peaked in September, while *A. flavus* was detected with a relatively low frequency (2%-15%) over the whole period of sampling. Other aspergilli were not constantly present. *A. versicolor* peaked in July (50% and 70%), while *A. fumigatus* was recovered with the highest frequency in November-December (17% and 30%). Frequency of indoor aeromycota correlates with outdoor aeromycota occurrence, except in the case of *Chrysonilia sitophila* which was never recovered from outdoor samples.

Table 1. Variations in concentration of airborne fungi in 2 sawmills (SM 1, SM 2) and outdoor air (ODA) in relation to temperature and relative humidity during sampling period

Sampling period	Concentration of airborne fungi (cfu/m^3)						Temperature (°C)**			Relative humidity (%)		
	SM 1		SM 2		ODA		SM 1	SM 2	ODA	SM 1	SM 2	ODA
	(mean ± SD)	Min-max	(mean ± SD)	Min-max	(mean ± SD)	Min-max						
April-May	6215±3192*,a	300-9600	2842±1971*	680-8180	530±83	640-660	19.9	17.1	22.7	41.8	48.0	41
July	7316±3849*,a	820-14400	4819±3264*	860-8780	710±256	480-1200	28.4	25.0	26.0	56.0	60.2	50.2
September	1868±1610*	380-4660	1851±2486*	160-8180	430±98	320-540	16.1	15.7	16.6	60.7	64.1	65
November-December	1696±1756*	160-5120	1706±1175*	620-5020	158±76	82-260	11.3	9.1	5	47.3	42.6	50.5
February	1754±1858*	160-6120	1793±1471*	620-7020	173±74	82-340	14.1	12.3	10.2	44.3	41.2	38

Mean cfu/m^3±SD represents average values from all plates taken at different sampling sites;
* ($p<0.001$) SM 1, SM 2 *vs* ODA;
[a] ($p<0.05$) SM 1 *vs* SM 2;
** p=0.017 (by Spearman correlation test) T *vs* cfu/m^3 (SM 1, SM 2, ODA).

Table 2. Frequency of isolation of aeromycota in 2 sawmills (SM 1, SM 2) and outdoor air (ODA) during sampling period

Airborne fungi	April-May (%)			July (%)			September (%)			November-December (%)			February (%)		
	SM 1	SM 2	ODA	SM 1	SM 2	ODA	SM 1	SM 2	ODA	SM 1	SM 2	ODA	SM 1	SM 2	ODA
Acremonium	8	-	25	-	-	25	-	-	-	8	8	12	-	-	-
Alternaria	30	16	50	36	30	75	27	7	50	-	-	-	3	3	-
Aspergillus flavus	8	2	8	11	7	-	13	13	12	8	15	12	8	8	8
A. fumigatus	8	-	-	-	-	-	13	12	-	17	30	30	4	4	12
A. versicolor	8	2	25	50	71	25	-	-	-	8	8	-	8	4	-
A. niger	71	65	25	43	50	50	20	47	37	17	15	25	15	15	25
Chrysonilia sitophila	96	62	-	68	56	-	40	33	-	54	46	-	8	8	-
Cladosporium	8	2	75	73	76	90	73	60	70	-	-	-	23	16	50
Epicoccum	4	7	12	20	7	75	27	27	37	-	-	-	7	10	-
Fusarium	4	7	-	-	7	-	-	-	-	-	-	-	-	-	-
Mucor	-	-	-	3	7	-	-	-	-	8	8	-	15	15	-
Nigrospora	8	10	-	4	2	-	-	-	-	-	-	-	-	-	-
Paecilomyces	50	69	75	43	50	25	53	47	37	83	100	100	8	15	25
Penicillium	50	73	75	100	100	75	80	80	57	92	100	100	92	92	100
Rhizopus	17	19	-	18	15	-	20	20	-	8	8	-	15	15	25
Trichoderma	8	19	25	21	26	-	40	33	-	25	36	-	15	8	-
Other fungi*	13	8	12	21	14	-	20	20	-	15	15	-	19	14	-

% represents positive samples in the total number of samples for each location;
** Auerobasidium, A. clavatus, Chaetomium, Phoma, Ulocladium* and unidentified fungi; - not detected.

Species of *Cladosporium* and *Alternaria* peaked in July (76% and 36%) in both sawmills, but both entities had a higher frequency outdoors (90% and 75%) and were not recovered from samples taken in November-December. Other identified fungal entities were not detected in more than one sampling period and occurred in 2-27% of sawmills samples, and in 12-75% of all outdoor samples.

Total airborne dust concentration. Employers from both sawmills provided data obtained during obligatory checking of working conditions within the sawmills, performed by licensed firms for protection at work. In both sawmills, the total airborne dust concentrations were below 1 mg/m^3.

Respiratory symptoms. The study included 35 workers from SM 1 and 61 workers form SM 2. Subjects significantly differed between the 2 sawmills regarding gender, age, working exposure and smoking index (Tab. 3). Although men prevailed in both sawmills, the proportion of females was significantly higher in SM 2 than in SM 1 (39% *vs* 6%, respectively; $p<0.0001$). Subjects from SM 1 were significantly older than subjects from SM 2 (40.3 *vs* 35.1 years, respectively; $p=0.029$). Working exposure in sawmills was generally short, but was significantly shorter for subjects from SM 1 than for subjects from SM 2 (median 3 *vs* 5 years, respectively; $p=0.005$). Although the proportions of current smokers were similar in both sawmills, the smoking index was significantly higher for smokers from SM 1 than for smokers from SM 2 (median 400 *vs* 165, respectively; $p=0.032$). Rhinitis, asthma and dry cough were most frequently recorded among analysed wood-processing workers (Fig. 2). Symptoms of rhinitis and asthma were more frequent in subjects from SM 1 than subjects from SM 2, with statistical significance reached for asthma symptoms (40% *vs* 23% of subjects with rhinitis, respectively, $p=0.077$; 29% *vs* 5% subjects with asthma, respectively, $p=0.003$). Dry cough, phlegm and chronic bronchitis were similarly recorded among subjects from SM 1 and SM 2 (26% *vs* 18% subjects with dry cough, respectively; 14% *vs* 18% subjects with phlegm, respectively; 11% *vs* 10% subjects with chronic bronchitis, respectively; $p>0.05$).

Table 3. Main characteristics of study subjects (N=96)

Subjects	SM 1	SM 2	P
N (men)	35 (33)	61 (37)	0.0001
Age (years), mean (SD)	40.3 (11.5)	35.1 (10.7)	0.029
Work exposure (years), median (IQ range)	3 (1-7)	5 (3-10)	0.005
Current smokers, N (%)	17 (49)	34 (56)	0.498
Smoking index, median (IQ range)	400 (240-500)	165 (42-300)	0.032
Subjects with positive SPT, N (%)	11 (33)	18 (30)	0.702
Atopic subjects, N (%)	8 (24)	8 (13)	0.171

SM – sawmill; Work exposure – duration of work exposure in wood-processing industry;
IQ – interquartile range;
Smoking index – number of cigarettes smoked per day x number of smoking years;
Subjects with positive SPT – subjects with one or more positive reactions in skin prick test to common inhalatory allergens;
Atopic subjects – subjects with positive skin prick test and symptoms of rhinitis and/or asthma;
p – statistical assessment of difference between subjects from SM1 and SM2.

Skin prick testing. The proportions of subjects with positive SPT to at least one common inhalatory allergen and subjects with atopy were similar in SM 1 and SM 2 (33% *vs* 30% subjects with positive SPT, respectively; 24% *vs* 13% subjects with atopy, respectively; $p>0.05$). The frequency of positive SPT to common inhalatory allergens among 94 skin prick tested workers did not differ significantly between sawmills, with the most prevalent reactions to dust mites *D. pteronyssinus, D. farinae* and *L. destructor* (21 workers, i.e. 22%) and *Artemisia vulgaris* pollen (9 workers, i.e. 10%). Positive SPT to grass pollen was present in 4%, to *Ambrosia*

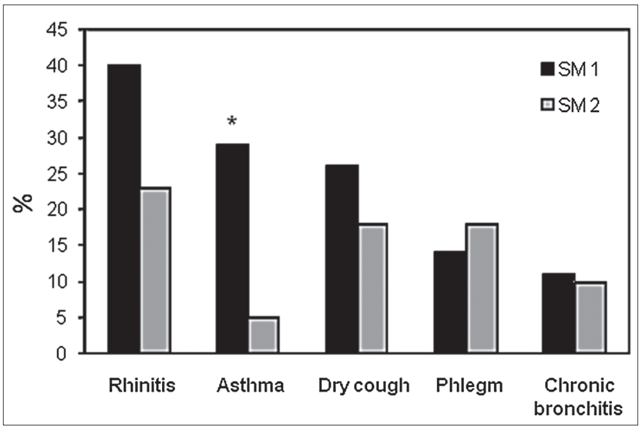

Figure 2. Prevalence of respiratory symptoms in subjects from the 2 sawmills.
*p=0.003 (by Fisher's exact test);
SM – sawmill;
Rhinitis – 2 or more upper respiratory symptoms (sneezing, rhinorrhea, nasal itching, nasal obstruction);
Phlegm – cough with expectoration;
Asthma – wheezing and/or dyspnea;
Chronic bronchitis – persistent cough and phlegm for more than 3 months per year, lasting more than 2 years.

elatior pollen in 4%, to birch pollen in 6% and to hazel pollen in 6% of workers. Only 2 workers were sensitized to cat epithelia (2%), and no positive reactions were found to dog epithelia. Regarding skin prick testing to moulds, no positive reactions to *Cladosporium herbarum, Alternaria alternata, Aspergillus niger,* and *Penicillium notatum* were found. Only one subject from SM 1 had positive SPT reaction to *Rhizopus nigricans.*

DISCUSSION

Average concentrations of airborne fungi showed that the air in the 2 Croatian sawmills were moderately loaded with fungi (1.7-7.3x10^3 cfu/m^3) compared to some other occupational environments, including composting facilities (mean 4.9x10^5-9.3x10^7 cfu/m^3), biofuel plants factory (mean 1.3x10^6 cfu/m^3), grain mills (mean 1x10^4 cfu/m^3) or poultry farms (mean 1.27-2.59x10^4 cfu/m^3) [4, 6, 8, 18]. The levels of airborne fungi found in the Croatian sawmills are comparable with those measured in Polish sawmills where average fungal concentrations were between 0.6-17.3x10^3 cfu/m^3, depending on the work site [19]. The presented study also showed that levels of airborne fungi were influenced by work site as well as season, showing a significant positive correlation with temperature. Considerably higher levels were recorded in April-May and July in both sawmills, compared to those obtained in other months of sampling, confirming earlier findings concerning seasonal differences in fungal air concentrations [20]. Levels hazardous for health (>10,000 cfu/ m^3) were found only in SM 1 during the summer. SM 1 had an airborne fungal burden that was approximately twice as high in the spring-summer period than SM 2. Also, higher levels of fungal air contamination near the sawmills were obtained in other months of sampling in SM 1 as compared to SM 2 (Fig. 1) These differences could be due to differences in the technology of wood processing between the two sawmills. A higher level of fungal contamination of timber can be expected if it was kept outdoors for a longer period, and/or due to poor wood dust suction at machine sites. The result of any aeromycological study is highly influenced by the method of sampling, type of sampler and collecting media, time of sampling, frequency of collection, location, and other factors [20]. The type of sampler used in this study had a high flow and high impact rate. Therefore, a short sampling time (30 sec) was used to avoid overloading the culture plates. However, some species present at a lower concentration, are probably overgrown with the dominant ones, and their presence and/or number might be underestimated.

In the presented study, *Penicillium* spp. followed by *Paecilomyces* spp. and *Chrysonilia sitophila* prevailed over the other identified fungal genera. *Penicillium* is one of the most prevalent fungal genera in many occupational environments [8]. The domination of *Penicillium* spp. was detected in sawmills in Poland and the USA that exploited hardwood such as oak and beech [19, 21]. Opplinger *et al.* [5] also reported the prevalence of penicillia in Swiss sawmills which processed Norwegian spruce, beech and silver fir. According to previous aeromycological studies, *Paecilomyces* spp. together with *Rhizopus* spp. was more abundant in sawmills that processed conifers such as pine [4, 8]. *Chrysonilia sitophila* (asexual state of *Neurospora sitophila*) degrades suberin (in cork), lignin and cellulose of wood. It was found as the dominant species in the air of cork stopper factories, and was airborne in wine cellars [22, 23]. Since it was detected in both sawmills in Croatia which processed oak, its presence could be attributed to the colonisation and degradation process of oak timbers. Also, it should be noted that studies conducted in sawmills in Switzerland, Poland, Lithuania and the USA [5, 8, 19, 21] did not report *Chrysonilia sitophila* among identified airborne fungi, which is probably related to the occurrence of *Chrysonilia* in warmer environments [24]. Other airborne fungi that were constantly present but with lower frequency were *A. niger* (15%-71%), *Trichoderma* spp. (8%-40%), *Rhizopus* spp. (8%-20%) and *A. flavus* (2%-15%). At the same time, *Trichoderma* spp. and *Rhizopus* spp. were detected in outdoor air only during one month of sampling. Their higher frequency in sawmills than outdoors is probably related to the water activity of wood timber and/or decaying processes. *Aspergillus fumigatus* and *A. versicolor* were not constantly present, neither indoors nor outdoors. Both *Aspergillus* and *Trichoderma* species were also found with lower frequency in sawmills in Lithuania and the USA [8, 21].

Opposite to our findings, in Polish sawmills, *A. fumigatus* was among the dominant mould species, but its high concentration was related to the debarking of pine wood. In the present study, *Cladosporium* and *Alternaria* species had a higher frequency outdoors than in sawmills, which is not surprising because these species are more related to conifers than hardwood [5, 21]. This observation is in line with our previous study in which measurable levels of Alt a 1 (main allergen of *A. alternata*) were not detected by the ELISA method in settled dust samples from sawmills processing predominantly hardwood species [25].

Studies investigating respiratory health in wood processing workers found nasal irritation (blocked nose, runny nose, sneezing), cough, symptoms of chronic bronchitis, asthma-like symptoms, and lung function cross-shift decline as the most consistent findings [4, 26, 27]. Exact causative factors and pathophysiological mechanisms underlying these respiratory impairments are still not fully understood. Wood processing workers are exposed to complex and variable mixtures of wood dust and microorganisms (bacteria, fungi) and it is hard to define which constituent of wood dust is responsible for respiratory impairment. Certain studies found correlations between respiratory symptoms and wood dust concentrations [28], and correlations of airborne fungi concentration with respiratory symptoms [29] or lung function decline [30] in wood processing workers. Several studies found that the work environment in sawmills is characterized with low to moderate inhalable dust concentrations, low endotoxin levels and high airborne fungi concentrations (>10,000 cfu/m^3) [5, 28, 29, 31, 32], suggesting that fungi should be considered as a dominant cause of respiratory disorders among sawmill workers [4]. In this study, total dust concentrations were low (<1 mg/m^3) in both sawmills, and airborne fungi concentrations were <10,000 cfu/m^3 in the majority of samples. Therefore, both dust and fungi concentrations were below threshold levels for the occurrence of respiratory impairments [4]. However, our previous study revealed endotoxin levels in the air of both sawmills >125 EU/m^3 (threshold level for occurrence of respiratory symptoms), suggesting Gram-negative bacteria as a wood dust constituent responsible for the development of respiratory impairments in workers from analyzed sawmills [33].

The most prevalent symptoms among sawmill workers were symptoms of rhinitis, asthma and dry cough, but their prevalence differed significantly between sawmills, mainly due to the differences between workers from the 2 sawmills. The higher prevalence of rhinitic and particularly asthmatic symptoms (including dry cough) in workers from SM 1 compared to workers from SM 2 could be attributed mainly to a greater proportion of atopic subjects and heavier smoking habit among the workers from SM 1. However, hazardous endotoxin levels, together with higher exposure to airborne fungi in SM 1 than in SM 2, should also be considered in the discussion of possible causes of respiratory impairment. Atopy – defined as the presence of both positive SPT to at least one common inhalatory allergen and symptoms of rhinitis and/or asthma [9], was present in workers from SM 2 in the same proportion as for the general population of Croatia (13% *vs* 12%, respectively) [34], while the proportion of atopic workers was somewhat greater in SM 1 (24%). Other studies also support the fact that atopic subjects are present among woodworkers in similar proportions (30), or even greater (35), than in the general populations, suggesting that the working environment in the wood processing industry is not hazardous enough to provoke "healthy worker effect" related to atopy, as opposed to some other occupational organic dust exposures, e.g. poultry farms [18].

On the other hand, there are some suggestions that atopy should be considered as risk factor for developing asthma in conditions of exposure to wood dust [36]. Additionally, the fluctuation of workers in the wood processing industry is high with often short duration of working exposures, as was also found in the presented study (median 3 and 5 years of employment in SM 1 and SM 2, respectively). It was suggested that besides socio-economic factors, the reason for such fluctuation could be the self-selection of workers due to health impairments [29].

Many fungal species, including *Alternaria, Cladosporium, Aspergillus, Penicillium, Rhizopus* and *Chrysonilia* spp., were defined as sources of allergens, inducers of IgE-mediated sensitization, and causes of atopic respiratory diseases like allergic rhinitis or asthma. Moreover, the main allergens were characterized for *Alternaria alternata, Aspergillus fumigatus* and *Cladosporium herbarum* [4, 12, 37]. Most studies that attempted to elucidate the pathophysiological pathways of respiratory impairments related to occupational airborne fungi exposure suggest, however, the involvement of non-allergic, neutrophilic inflammation [4, 12]. IgE-mediated sensitization to fungi (*Cladosporium herbarum, Alternaria alternata, Aspergillus niger, Penicillium notatum,* and *Rhizopus nigricans*) was practically absent in the tested sawmill workers in the present study. According to exposure assessment, sensitization to at least *Penicillium* spp. was to be expected, but our results did not suggest IgE-mediated sensitization to these or other tested fungi to be relevant as a mechanism of respiratory impairment in sawmill workers. The same results were found for Norwegian sawmill workers regarding exposure to *Rhizopus microsporus* [38]. However, besides exposure to moderate airborne fungi levels, short employment duration of the tested sawmill workers, and commercial allergens used for SPT, should be considered in the absence of IgE-mediated sensitization to fungi in this study. Positive skin reactions to fungal allergens could probably occur more frequently if the testing was performed with allergens produced from fungal strains isolated from the air of the analysed sawmills.

CONCLUSIONS

In taking all the data together, this study confirms earlier findings that the type of wood has great influence on aeromycota composition in sawmills. Throughout the year, Croatian sawmill workers who process mainly hard wood and fir wood are more exposed to species of *Penicillium*, *Paecilomyces* and *Chrysonilia*. Health hazardous levels of airborne fungi (above 10^4/m^3) were related to saw working sites and were season-dependent, i.e. measured only during the summer, and present only in one sawmill (SM 1). Such airborne fungi levels were not related to the development of IgE-mediated sensitization to *Penicillium, Aspergillus, Rhizopus, Alternaria* and *Cladosporium spp.* in exposed workers, even in atopics. Atopy was present among woodworkers in similar or greater proportions to that among the general population, suggesting that the working environment in the wood-processing industry is not selective for atopic workers.

Acknowledgements

This work was financially supported by the Ministry of Science, Education and Sports of the Republic of Croatia (Grant Nos. 006-0061117-1242 and 022-0222411-2410).

REFERENCES

1. Abdel Hameed AA, Khoder MI, Farag SA. Organic dust and gaseous contaminants at wood working shops. J Environ Monit. 2000; 2(1): 73-76.
2. Alwis KU, Mandryk J, Hocking AD. Exposure to biohazards in wood dust: bacteria, fungi, endotoxins, and (1→3)-beta-D-glucans. Appl Occup Environ Hyg. 1999; 14(9): 598-608.
3. Krysińska-Traczyk E, Skórska C, Cholewa G, Sitkowska G, Milanowski J, Dutkiewicz J. Exposure to airborne microorganisms in furniture factories. Ann Agric Environ Med. 2002; 9(1): 85-90.
4. Eduard W. Fungal spores: A critical review of the toxicological and epidemiological evidence as a basis for occupational exposure limit setting. Crit Rev Toxicol. 2009; 39(10): 799-864.

5. Opplinger A, Rusca S, Charriere N, Duc TV, Droz PO. Assessment of bioaerosols and inhalable dust exposure in Swiss sawmills. Ann Occup Hyg. 2005; 49(5): 385-391.
6. Madsen AM. Exposure to airborne microbial components in autumn and spring during work at Danish biofuel plants. Ann Occup Hyg. 2006; 50(8): 821-831.
7. Čavlović A, Lučić Beljo R, Ištvanić J. Exposure to wood dust in Croatian woodworking industry. Wood Res. 2009; 54(3): 109-116.
8. Lugauskas A, Krikštaponis A, Šveistyte L. Airborne fungi in industrial environments – potential agents of respiratory diseases. Ann Agric Environ Med. 2004; 11(1): 19-25.
9. Johansson SGO, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF et al. Revised nomenclature for allergy for global use: Report of the nomenclature review committee of the World allergy organization, October 2003. J Allergy Clin Immunol. 2004; 113(5): 832-836.
10. Monsó E, Magarolas R, Badorrey I, Radon K, Nowak D, Morera J. Occupational asthma in greenhouse flower and ornamental plant growers. Am J Respir Crit Care Med. 2002; 165(7): 954-960.
11. Zhang Y, Chen J, Chen Y, Dong J, Wei Q, Lou J. Environmental mycological study and allergic respiratory disease among tobacco processing workers. J Occup Health. 2005; 47(2): 181-187.
12. Douwes J, Thorne P, Pearce N, Heederik D. Bioaerosol health effects and esposure assessment: progress and prospects. Ann Occup Hyg. 2003; 47(3): 187-200.
13. Feller W. An Introduction to the probability theory and its application, 175. John Wiley and Sons Inc, New York, 1950.
14. de Hoog GS, Guarro J, Gene J, Figueras MJ. Atlas of clinical fungi. 2nd ed. Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili, Baard-Delft, 2000.
15. Pitt JI, Hocking AD. Fungi and food spoilage. Springer Science+Business Media LLC, New York, 2009.
16. Rylander R, Peterson Y, Donham KJ. Questionnaire evaluating organic dust exposure. Am J Ind Med. 1990; 17(1): 121-126.
17. Dreborg S, Frew A. Position paper: Allergen standardization and skin tests. Allergy 1993; 48(14 Suppl): 49-82.
18. Rimac D, Macan J, Varnai VM, Vučemilo M, Matković K, Prester L et al. Exposure to poultry dust and health effects in poultry workers: impact of mould and mite allergens. Int Arch Occup Environ Health. 2010; 83(1): 9-19.
19. Dutkiewicz J, Krysińska-Traczyk E, Prażmo Z, Skórska C, Sitkowska J. Exposure to airborne microorganisms in Polish sawmills. Ann Agric Environ Med. 2001; 8(1): 71-80.
20. Šegvić Klarić M, Pepeljnjak S. A year-round aeromycological study in Zagreb area, Croatia. Ann Agric Environ Med. 2006; 13(1): 55–64.
21. Simeray J, Mandin D, Chaumont JP. An aeromycological study of sawmills: effects of type of installation and timber on mycoflora and inhalation hazards for workers. Int Biodeter Biodegr. 1997; 40(1): 1 l-17.
22. Haas D, Galler H, Habib J, Melkes A, Schlacher R, Buzina W et al. Concentrations of viable airborne fungal spores and trichloroanisole in wine cellars. Int J Food Microbiol. 2010; 144(1): 126-132.
23. Oliveira AC, Peres CM, Correia Pires JM, Silva Pereira C, Vitorino S, Figueiredo Marques JJ et al. Cork stoppers industry: defining appropriate mould colonization. Microbiol Res. 2003; 158(2): 117–124.
24. San Romão MV. Biological diversity preservation as a challenge - the role of microbiological cork colonisation a short review. Ciência Téc Vitiv. 2009; 24(2): 81-90.
25. Prester Lj, Macan J. Determination of Alt a 1 *(Alternaria alternata)* in poultry farms and a sawmill using ELISA. Med Mycol. 2010; 48(2): 298-302.
26. Jacobsen G, Schaumburg I, Sigsgaard T, Schlünssen V. Non-malignant respiratory diseases and occupational exposure to wood dust. Part I. Fresh wood and mixed wood industry. Ann Agric Environ Med. 2010; 17(1): 15-28.
27. Jacobsen G, Schaumburg I, Sigsgaard T, Schlünssen V. Non-malignant respiratory diseases and occupational exposure to wood dust. Part II. Dry wood industry. Ann Agric Environ Med. 2010; 17(1): 15-28.
28. Mandryk J, Udeni Alwis K, Hocking AD. Work-related symptoms and dose-response relationships for personal exposures and pulmonary function among woodworkers. Am J Ind Med. 1999; 35(5): 481-490.
29. Rusca S, Charriere N, Droz PO, Opplinger A. Effects of bioaerosol exposure on work-related symptoms among Swiss sawmill workers. Int Arch Occup Environ Health. 2008; 81(4): 415-421.
30. Dahlqvist M, Johard U, Alexandersson R, Bergström B, Ekholm U, Eklund A et al. Lung function and precipitating antibodies in low exposed wood trimmers in Sweden. Am J Ind Med. 1992; 21(4): 549-559.
31. Dennekamp M, Demers PA, Bartlett K, Davies HW, Teschke K. Endotoxin exposure among softwood lumber mill workers in the Canadian province of British Columbia. Ann Agric Environ Med. 1999; 6(2): 141-146.
32. Mandryk J, Udeni Alwis K, Hocking AD. Effects of personal exposures on pulmonary function and work-related symptoms among sawmill workers. Ann Occup Hyg. 2000; 44(4): 281-289.
33. Sabolić Pipinić I, Varnai VM, Beljo Lučić R, Čavlović A, Prester LJ, Orct T et al. Endotoxin exposure assessment in wood-processing industry: airborne versus settled dust levels. Arh Hig Rada Toksikol. 2010; 61(2): 161-166.
34. Macan J, Varnai VM, Maloča I, Kanceljak-Macan B. Increasing trend in atopy markers prevalence in a Croatian adult population between 1985 and 1999. Clin Exp Allergy. 2007; 37(12): 1756-1763.
35. Douwes J, McLean D, Slater T, Travier N, Cheng S, Pearce N: Pine dust, atopy and lung function: a cross-sectional study in sawmill workers. Eur Respir J 2006, 28, 791-798.
36. Schlünssen V, Schaumburg I, Heederik D, Taudorf E, Sigsgaard T. Indices of asthma among atopic and non-atopic woodworkers. Occup Environ Med. 2004; 61(6): 504-511.
37. Francuz B, Yera H, Geraut L, Bensefa-Colas L, Nghiem ZH, Choudat D. Occupational asthma induced by *Chrysonilia sitophila* in a worker exposed to coffee grounds. Clin Vaccine Immunol. 2010; 17(10): 1645-1646.
38. Rydjord B, Eduard W, Stensby B, Sandven P, Michaelsen TE, Wiker HG. Antibody response to long-term and high-dose mold-exposed sawmill workers. Scand J Immunol. 2007; 66(6): 711-718.