MICROBIAL CONTAMINANTS OF STORED TIMBER AS POTENTIAL RESPIRATORY HAZARDS FOR SAWMILL WORKERS

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INTRODUCTION

Occupational exposure to wood dust may be a cause of respiratory diseases such as hypersensitivity pneumonitis (allergic alveolitis),[7,12,23,26] asthma[8] and chronic obstructive lung disease (COLD).[2] The etiology of these diseases is not fully known and both the allergenic and/or toxic constituents of wood tissue itself and the substances produced by microorganisms developing in wood have been suggested as potential agents.[8,10,22,27] Many species of allergenic and/or toxic molds developing on wood (*Alternaria tenuis, Aspergillus fumigatus, Cryptostroma corticale, Mucor spp., Paecilomyces spp., Penicillium spp., Rhizopus spp.)* have been described as causative agents of pulmonary diseases in woodworkers.[6,7,8,12,23,24,25,26] The role of bacterial factors in wood-associated diseases was studied to a lesser extent.[10,22] It has been reported that woodworkers may be exposed to notable amounts of gram-negative bacteria and endotoxin.[1,4,25]

The aim of this study was to extend the knowledge of the potential respiratory risk of woodworkers to wood-inhabiting microorganisms by quantitative and qualitative determination of the microflora of stored timber logs scheduled for processing in a sawmill.

MATERIAL AND METHODS

Two series of microbiological wood samples were taken in August and October of the year 1987 from timber logs stored on the lumber yard at a sawmill in Kingwood, West Virginia. The logs had been stored for a period of 4–6 weeks and did not show any apparent signs of decay. At each sampling time, samples were taken from a log of each of the following species: American basswood (*Tilia americana L.*), black cherry (Prunus serotina Ehrh.), black locust (*Robinia pseudoaccacia L.*), red oak (*Quercus coccinea Muenchh.*), soft maple (*Acer saccharinum L.*) and white poplar (Populus alba L.). From each log, one sample was taken from the heartwood (by boring from the transverse section), one from the sapwood (by boring from the transverse section) and one from the bark (by centripetal boring).

The wood samples were collected with a novel "drill and collect" device (model #2) for quantification of microorganisms in wood.[5] This is a manually operated drilling device in which a combined action of a twist boring bit and a spring-containing mobile ring collects the pulverized wood into a sterile flask attached beneath the bit in a one-step sterile process. The wood surface to be sampled was first sterilized by wiping with 70% propanol and "Clorox" (a commercial 5.25% sodium hypochlorite solution) and then an average sample was taken by multiple boring (5–7 times) in a circle up to 3 cm in diameter.

The concentrations of bacteria and fungi in the wood samples were determined by dilution plating. Aliquots of 200 mg of each sample were suspended in 20 ml of sterile phosphate buffered saline (Sigma Chemical Co., St. Louis, MO) containing 0.1% (v/v) Tween 80 (Fisher Scientific Co., Fair Lawn, NJ) and, after vigorous shaking, serial 10-fold dilutions were made up to 10^{-6}. The 0.1 ml aliquots of each dilution were spread on duplicate sets of the following agar media: (i) sheep blood agar for total aerobic bacteria, (ii) eosin methylene blue agar (EMB agar; Difco Lab., Detroit, MI) for gram-negative bacteria, (iii) half-strength tryptic soya agar (Difco) for thermophilic actinomycetes, (iv) rose bengal streptomycin agar (RBS) for total fungi, and (v) yeast malt agar for yeasts. The blood agar and EMB plates were incubated for 48 hrs at 35°C, the tryptic soya plates for 120 hrs at 55°C, and the RBS and yeast malt plates for 96 hrs at 28°C.

Following incubation, bacterial colonies were counted and differentiated on the basis of colony morphology, Gram reaction, and biochemical reactions. The gram-positive isolates were identified according to Bergey's Manual.[21] The gram-negative isolates were identified with the API^{R} Systems 20 E (for enterobacteria) and NFT (for non-fermenting bacteria) (API Analytab Products, Plainview, NY), using supplementary biochemical tests selected according to Bergey's Manual[14] and API^{R} Systems recommendations. Mold colonies were counted and differentiated on the basis of morphological properties. Representative yeast colonies were isolated and differentiated on the basis of morphological and biochemical properties.[13] Final results for microbial concentrations were reported in terms of the colony forming units (cfu) in one gram of ground wood.

For endotoxin determination, 100 mg portions of the wood samples were extracted with 5 ml of sterile non-pyrogenic water (Travenol Laboratories Inc., Deerfield, IL) by rocking for 60 min. at room temperature. The suspension was centrifuged at 1000 g for 10 minutes to remove particulate debris, and the supernatant fluid was separated for further

analysis. Quantification of gram-negative bacterial endotoxin content was performed in duplicate by a quantitative chromogenic modification of the Limulus amebocyte lysate test (QCL-1000; Whittaker Bioproducts, Walkersville, MA). Results were reported in terms of Endotoxin Units (EU) in one gram of ground wood.

The Students' t-test for matched pairs, test for linear regression and chi-square test were used for statistical evaluation of the results.

RESULTS

The concentration of microorganisms and endotoxin varied significantly with the kind of wood examined ($P<0.001$). As shown in Figures 1–6, the highest levels of bacteria, fungi and endotoxin were found in the wood samples from logs of American basswood and black locust (10^3–10^8 cfu/gm, 10^2–10^7 cfu/gm and 10^4–10^6 EU/gm, respectively). The levels were lower in the logs of soft maple and black cherry (0–10^6 cfu/gm, 0–10^7 cfu/gm and 10^1–10^4 EU/gm, respectively) and lowest in the logs of white poplar and red oak (0–10^4 cfu/gm, 0–10^5 cfu/gm and 10^0–10^5 EU/gm, respectively).

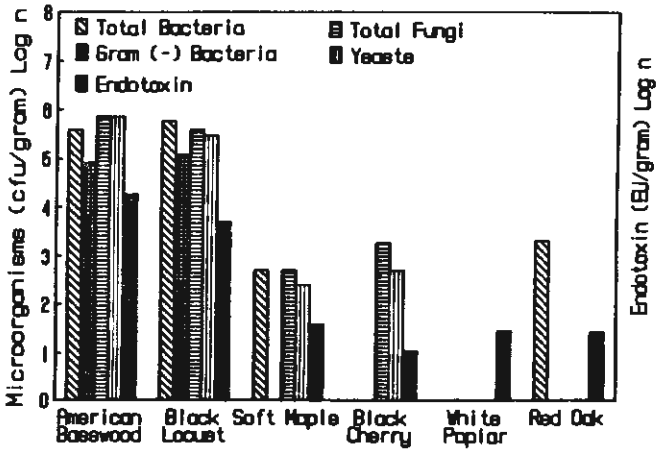

Figure 1. Concentrations of bacteria, fungi and endotoxin in the samples of heartwood collected in August, 1987.

High concentrations of bacteria, fungi and endotoxin were found in all the examined kinds of wood tissue: heartwood (Figures 1–2), sapwood (Figures 3–4) and bark (Figures 5–6). No significant differences were noted between the contamination rates in August and October ($P>0.05$).

In most of the samples of heartwood and sapwood, gram-negative bacteria dominated the total bacteria flora. Except for two cases (Figure 5), this was not observed in the bark samples. In bark samples taken in October, viable gram-negative bacteria were absent completely and the very high level of bacteria found in the bark of the black locust was due to the presence of large numbers of spore-forming bacilli (Figure 6). For each kind of wood tissue (heartwood, sapwood and bark) a significant correlation has been found between the concentrations of gram-negative bacteria and endotoxin ($P<0.05$). No thermophilic actinomycetes were found in the examined wood samples.

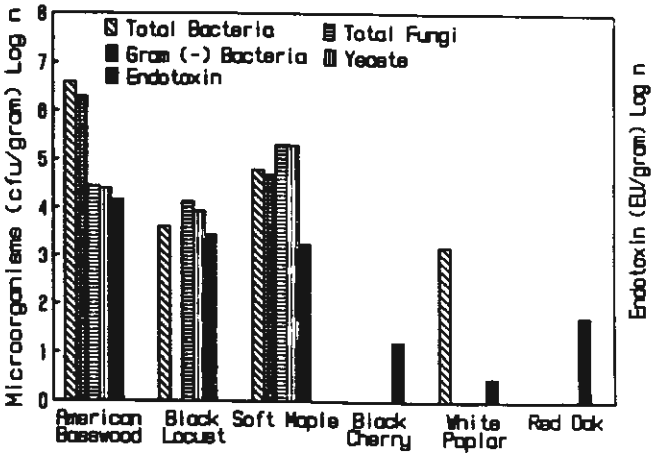

Figure 2. Concentrations of bacteria, fungi and endotoxin in the samples of heartwood collected in October, 1987.

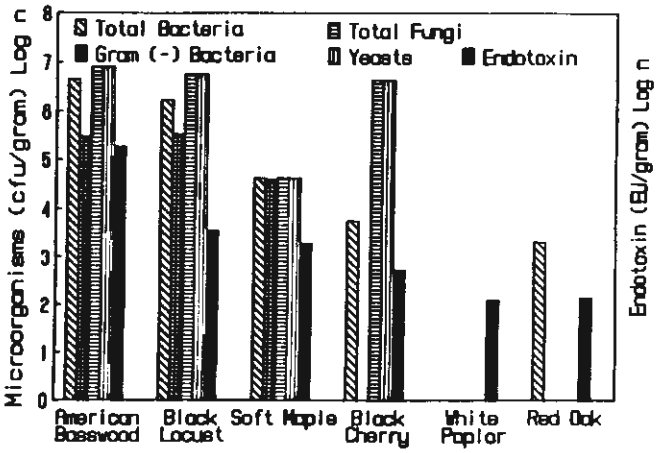

Figure 3. Concentrations of bacteria, fungi and endotoxin in the samples of sapwood collected in August, 1987.

Twelve species and/or genera of gram-negative bacteria and seven genera of gram-positive bacteria were found in the wood samples (Table I). The gram-negative flora comprised five fermentative species (belonging to the *Enterobacteriaceae* family) which, in most cases, were associated with the sapwood and seven non-fermentative species (mostly of the genus *Pseudomonas)* which were mostly associated with the heartwood. Among the gram-positive bacteria, the most common organisms were endospore-forming bacteria of the genus *Bacillus* and coryneform bacteria belonging to the genera *Arthrobacter, Brevibacterium, Corynebacterium* and *Microbacterium.*

In all kinds of wood samples examined, yeasts were the predominant fungi observed (Figures 1–6, Table II) and the

Table I
Species of Bacteria Occurring in Wood Samples

Name of the species	Heartwood	Sapwood	Bark	Maximal concentration (X 10^5 cfu/gram)
GRAM-NEGATIVE BACTERIA				
Fermentative				
Citrobacter freundii	+ (B, M)	++ (B), + (M)		0.10 (Sapwood, B)
Enterobacter agglomerans	++ (B), + (M)	+++ (B), + (M)	++ (B, L)	3.00 (Sapwood, B)
Enterobacter cloacae		++ (M)		0.38 (Sapwood, M)
Klebsiella sp.		+++ (B), + (M)		1.45 (Sapwood, B)
Serratia rubidaea	++ (L)	++ (L)		0.41 (Sapwood, L)
Non-fermentative				
Acinetobacter calcoaceticus	+ (M)		+++ (L)	30.50 (Bark, L)
Agrobacterium radiobacter	+++ (L)	+++ (L)		2.64 (Sapwood, L)
Pseudomonas fluorescens	+ (M)			0.07 (Heartwood, M)
Pseudomonas maltophilia	+ (M)			0.02 (Heartwood, M)
Pseudomonas oryzihabitans	+++ (B)			15.10 (Heartwood, B)
Pseudomonas putida	+++ (B), ++ (M)	+ (B)		3.84 (Heartwood, B)
Pseudomonas stutzeri	++ (B), + (M)	+ (M)		0.45 (Heartwood, B)
GRAM-POSITIVE BACTERIA				
Bacillus spp.	+++ (B), ++ (L), + (M)	+++ (L), + (B, M)	+++ (L, M), ++ (B), + (C, O, P)	154.00 (Bark, L)
Coryneform bacteria*	+++ (B, L), + (P)	+++ (B, L) + (C, M, O)	+++ (L), ++ (B, M), + (C, P)	20.30 (Heartwood, B)
Staphylococcus spp.	+ (M, O, P)	+++ (L), + (O)	+ (P)	5.00 (Sapwood, L)
Streptomyces spp.		+++ (L)	++ (L), + (B)	2.51 (Sapwood, L)

B = American Basswood M = Soft Maple + = occurred in concentration below 1 x 10^4 cfu/gram *Comprise: Arthrobacter spp., Brevibacterium spp. Corynebacterium spp.,

C = Black Cherry O = Red Oak ++ = occurred in concentration 1 x10^4 - 1 x 10^5 cfu/gram

L = Black Locust P = White Poplar +++ = occurred in concentration over 10^5 cfu/gram

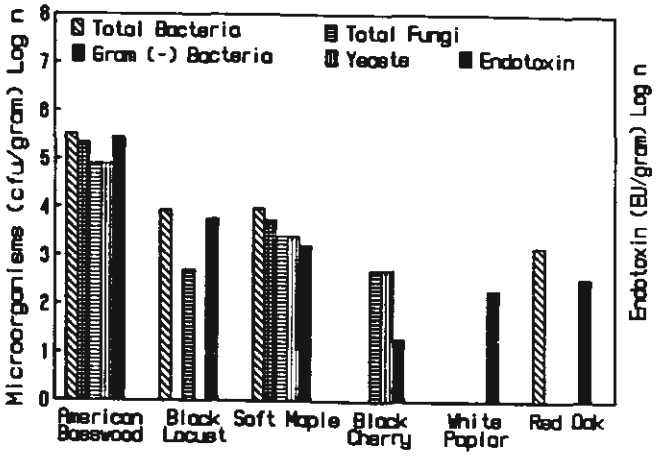

Figure 4. Concentrations of bacteria, fungi and endotoxin in the samples of sapwood collected in October, 1987.

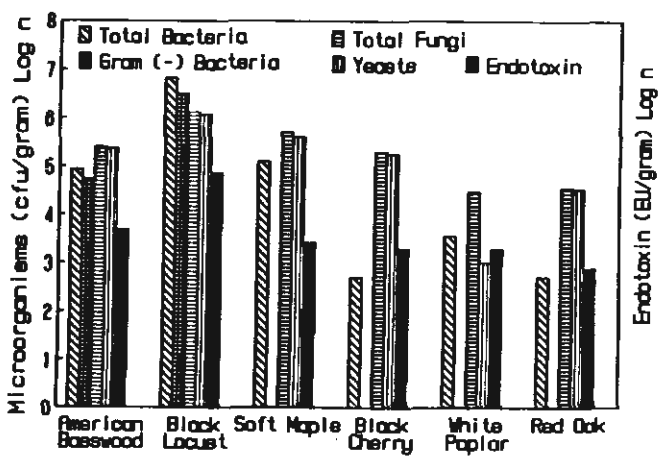

Figure 5. Concentrations of bacteria, fungi and endotoxin in the samples of bark collected in August, 1987.

Table II
Fungi Occurring in Wood Samples

Organism	Heartwood	Sapwood	Bark	Maximal concentration (X 10^5 cfu/gram)
DBB- yeasts[a]	+++ (B,L), ++ (M) + (C)	+++ (B,C,L), ++(M)	+++ (B,L,M), ++ (C,O), + (P)	78.35 (Sapwood, B)
DBB+ yeasts[b]	++ (B,L,M)	+++ (B), ++ (C,L)	++ (B,C,L,O)	1.45 (Sapwood, B)
Acremonium sp.	++ (L), + (B)	++ (L)	+++ (M), + (B,P)	14.00 (Bark, M)
Oidiodendron sp.			++ (C,M)	0.79 (Bark, M)
Penicillium sp.	++ (L)	++ (B,L)	++ (L,P), + (B,C,O)	0.72 (Bark, L)
Trichoderma sp.		++ (C)	++ (B,L)	0.49 (Bark, L)
Nonsporulating	+ (B,C,M)		++ (L,M), + (B,O,P)	0.26 (Bark, M)

[a]Negative reaction with Diazonium Blue B (DBB); presumptive Ascomycetes and their anamorphs (includes Candida zeylanoides, other undetermined Candida spp., and Hansenula silvicola.

[b]Positive reaction with DBB; presumptive Basidiomycetes and their anamorphs (includes undetermined Candida spp., Cryptococcus laurentii, and Rhodotorula glutinis).

B = American Basswood M = Soft Maple + = occurred in concentration below 1 x 10^4 cfu/gram
C = Black Cherry O = Red Oak ++ = occurred in concentration 1 x 10^4 - 1 x 10^5 cfu/gram
L = Black Locust P = White Poplar +++ = occurred in concentration over 10^5 cfu/gram

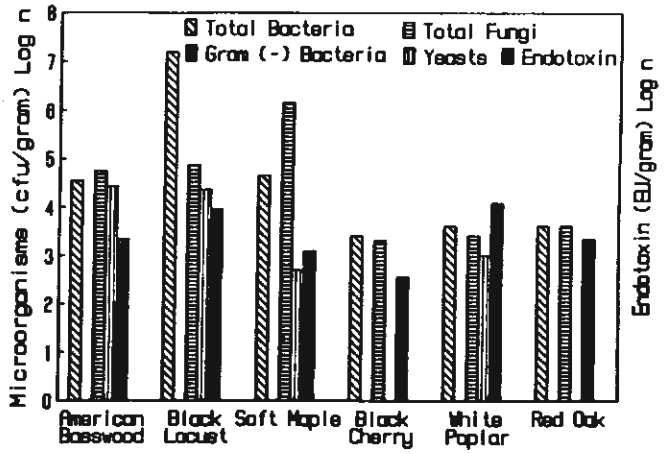

Figure 6. Concentrations of bacteria, fungi and endotoxin in the samples of bark collected in October, 1987.

most numerous among them were presumptive Ascomycetes and their anamorphs, i.e., they gave a negative reaction with Diazonium Blue B (DBB).[13] Yeast fungi tended to be found in the greatest numbers in the sapwood. Species of yeast isolated include undetermined *Candida spp.* (include both DBB+ and DBB− species), *Candida zeylanoides, Cryptococcus laurentii, Hansenula silvicola and Rhodotorula glutinis*.

Molds found in these samples included *Acremonium (Cephalosporium sp., Alternaria sp., Aspergillus fumigatus, Aureobasidium pullulans, Bispora sp., Cladosporium sp., Mortierella sp.,* and *Trichoderma sp.* as well a number of fungi that could not be identified because of their failure to sporulate. The molds that occurred in the greatest numbers were *Acremonium sp., Oidiodendron sp., Penicillium sp.,* and *Trichoderma sp.*. The highest numbers of molds were found in the bark.

DISCUSSION

The levels of microorganisms and endotoxin in timber logs showed notable variation depending on the species of the tree. The concentrations of bacteria and fungi in the most contaminated wood species (basswood, locust) exceeded the level of 10^6 cfu/gm, and were comparable to the values reported for certain organic dusts related to harmful respiratory effects in workers.[3]

The concentration of endotoxin in the wood reached, in many cases, a level of 10^5–10^6 EU/gm which corresponds to the values found in organic materials (grain, silage, mushroom farm pre-flush) associated with the cases of respiratory disorders in exposed workers.[17] This finding is in agreement with the fact that some of the wood samples contained high concentrations of gram-negative bacteria. Among these bacteria were the species *(Enterobacter agglomerans, Klebsiella spp., Pseudomonas putida)* which are known producers of biologically active endotoxin that can cause pulmonary

injury through non-specific stimulation of alveolar macrophages.[19]

The occurrence of high concentrations of fungi in the wood presents another factor of potential respiratory risk for sawmill workers. The *Penicillium* species that were frequently isolated in this study have been reported as a source of the pathogenic respiratory allergens.[6,24] Another potentially pathogenic species are *Aspergillus fumigatus* and *Aureobasidium pullulans*.[8,10,22]

The data conform to some earlier reports on the occurrence of bacteria and fungi in the wood[9,15,16,18] The composition of the microflora of examined logs, characterized by the prevalence of yeasts and gram-negative bacteria indicates that it was in the stage of "pioneer colonization" which precedes the stage of wood decay by brown rot and white rot fungi.[11,20]

The main conclusion from this preliminary study is that some kinds of apparently not decayed timber stored for processing in sawmill contain very high concentrations of "pioneer" microorganisms and their toxins. These organisms may cause respiratory disorders in the woodworkers if inhaled with the sawdust. Although not defined by the current study, the potential problems associated with microbiologically contaminated woods are intriguing and require further research.

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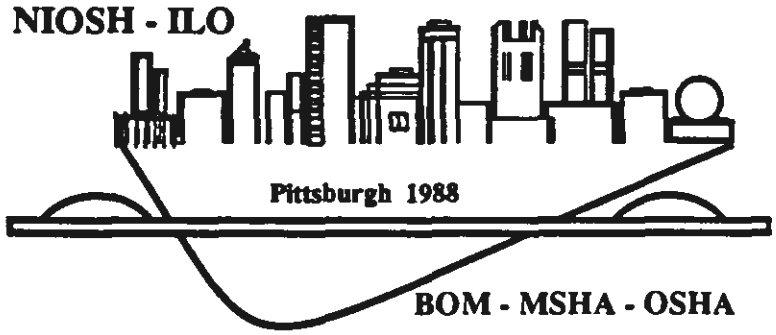

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