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Review

Microbial Occupational Exposure Assessments in Sawmills—A Review

Marta Dias [1,2,3], Bianca Gomes [3], Renata Cervantes [3], Pedro Pena [3], Susana Viegas [1,2,3] and Carla Viegas [1,2,3,*]

1 NOVA National School of Public Health, Public Health Research Centre, Universidade Nova de Lisboa, 1099-085 Lisbon, Portugal; marta.dias@estesl.ipl.pt (M.D.); susana.viegas@ensp.unl.pt (S.V.)
2 Comprehensive Health Research Center (CHRC), NOVA Medical School, Universidade NOVA de Lisboa, 1169-056 Lisbon, Portugal
3 H&TRC—Health & Technology Research Center, ESTeSL—Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa, 1990-096 Lisbon, Portugal; bianca.gomes@estesl.ipl.pt (B.G.); renata.cervantes@estesl.ipl.pt (R.C.); pedro_migpena@hotmail.com (P.P.)
* Correspondence: carla.viegas@estesl.ipl.pt

Abstract: The composition of airborne microflora in sawmills may vary to a great degree depending on the kind of timber being processed and the technology of production being used. Cases of allergy alveolitis and asthma have been reported in woodworkers who were exposed to wood dust largely infected with microorganisms. The aim of this review article is to identify studies where the microbial occupational exposure assessment was performed in sawmills and the characteristics of the contamination found, as well as to identify which sampling methods and assays were applied. This study reports the search of available data published regarding microbial occupational exposure assessment in environmental samples from sawmills, following the Preferred Reporting Items for Systematic Reviews (PRISMA) methodology. The most used sampling method was air sampling, impaction being the most common method. Regarding analytical procedures for microbial characterization, morphological identification of fungi and bacteria was the most frequent approach. Screening for fungal susceptibility to azoles was performed in two studies and four studies applied molecular tools. Regarding microbial contamination, high fungal levels were frequent, as well as high bacteria levels. Fungal identification evidenced Penicillium as the most frequent genera followed by Aspergillus sp. Mycotoxins were not assessed in any of the analyzed studies. Microbial occupational exposure assessment in sawmills is crucial to allow this risk characterization and management.

Keywords: occupational exposure; exposure assessment; sawmills; woodworkers; azole resistance; microbial contamination

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1. Introduction

Globally, the sawmill market is primarily driven by rising construction demand, which accounts for roughly 73.48 percent of total downstream consumption of sawmill in the world. Softwood and hardwood are the two types of sawmill raw materials. Its downstream use is diverse, and recently, building and furniture have gained prominence in a variety of sawmill areas [1].

Workers in sawmill industry may be exposed to allergic, carcinogenic, and immunotoxic agents, comprising wood derivatives (e.g., terpenes, resin acids) as well microorganisms that grow on timber (bacteria and fungi) and their products (endotoxins and mycotoxins) known as potential causative agents of health effects [2–8]. Exposure can result in decreased lung function, bronchial hyperresponsiveness, and a variety of disorders such as organic dust toxic syndrome (ODTS), allergic alveolitis, asthma, chronic bronchitis, rhinitis, mucous membrane irritation (MMI), contact dermatitis, and nasal cancer [9–18]. The majority of the negative effects generated by microorganisms linked with wood dust have an immunological basis.

The most well-known are those produced by fungi, which may thrive in the right conditions on stored wood products (planks, chips) as a secondary wood infection [18].

Inhaling large amounts of spores and mycelial fragments of *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Paecilomyces* sp., *Mucor* sp. and other fungi can result in a strong antibody response and respiratory disorders, most commonly allergic alveolitis (wood trimmer's disease) or organic dust toxic syndrome in exposed workers [18–27]. Cases of allergy alveolitis and asthma have been reported in woodworkers who were exposed to wood dust largely infected with fungi during logging, debarking, and sawing tasks [18].

The composition of airborne microflora in sawmills may vary to a great degree depending on the kind of timber being processed and the technology of production [8,15,16,28]. In wood processing, preservation, and maintenance azole fungicides are used for the protection of spruce and pine fields [28,29]. To protect wood from wood-destroying basidiomycete fungus, sawmills, particularly those working with resinous timbers, typically use azole fungicides. This fungus can induce deterioration or blueing of wood, rendering it useless [28,30]. Propiconazole and tebuconazole are the most common azole compounds found in sawmills. In fact, these two compounds are among the five 14-demethylase inhibitors (DMIs) linked to clinical azoles and contributing to the rise in azole antifungal resistance [28,30–32]. Furthermore, *Aspergillus* section *Fumigati* azole antifungal resistance was already reported in this environment [28,29].

Portugal's social and economic history is inextricably related to the products of the forest, where national economic organizations are world leaders in the production and trading of forest products [33]. Regarding the sawmill industry in Portugal, 2250 million euros were made with exportations in 2020, there were 8700 companies reported in the wood industry in 2019 and, consequently, about 56,000 workers account for this sector workforce [34].

Due to the lack of studies in Portuguese sawmills this study aimed to perform a systematic review to provide a broad overview of the state of art in the developed subject, describing the microbiological contamination reported in previous studies developed in sawmills and indicating which parameters and methods were applied to perform the microbial occupational exposure assessment in this setting. These study results will contribute to a sampling and analyses protocol proposal aiming to assess the occupational exposure to microbial contamination is this specific occupational environment.

2. Materials and Methods

2.1. Registration

The Preferred Reporting Items for Systematic Reviews (PRISMA) checklist [35] was completed (Supplementary Materials Table S1).

2.2. Search Strategy, Inclusion and Exclusion Criteria

This study reports the search of available data published between the period of 1 January 2000 and 30 September 2021. The search terms aimed to identify studies in microbial occupational exposure assessments, selecting studies on sawmills that included the terms "occupational exposure", "sawmills", with English as the chosen language. The databases chosen were PubMed, Scopus, Web of Science (WoS) and other sources, following the PRISMA methodology. This search strategy identified 441 papers in all databases. Articles that did not fulfil the inclusion criteria were not subjected to additional review (but some of them were used for introduction and discussion sections) (Table 1).

2.3. Studies Selection and Data Extraction

The selection of the articles was performed through Rayyan, which is a free web-tool that greatly speeds up the process of screening and selecting papers for academics working on systematic reviews, in three rounds by three investigators (MD, BG, and RC). The first round consisted of a screening of all titles to exclude papers that were duplicated or unrelated to the subject, and then the included added to Rayyan for further analysis. The second round consisted of a screening of all abstracts. In the third round, the full texts of all potentially relevant studies were reviewed considering the inclusion and exclusion criteria. Potential divergences in the selection of the study were discussed and ultimately resolved by the remaining investigators (CV and SV). Data extraction was performed by two investigators (BG and RG) and reviewed by another (MD). The following information was manually extracted: (1) Database, (2) Title, (3) Country, (4) Occupational Environment, (5) Sampling Methods, (6) Analytical Methods, (7) Main Findings, and (8) References.

Table 1. Inclusion and exclusion criteria in the articles selected.

Inclusion Criteria	Exclusion Criteria
Articles published in the English language;	Articles published in other languages
Articles published from 1 January 2000 to 30 September 2021	Articles published prior to 2000
Articles reporting findings from any country	
Articles related to microbial exposure assessment in sawmills	Articles related exclusively to biologic samples from workers or without mention microbial exposure.
Original scientific articles on the topic	Abstracts of congress, reports, reviews/state of the art articles

2.4. Quality Assessment

The assessment of the risk of bias was performed by two investigators (MD and CV). Within each study, we evaluated the risk of bias across three parameters divided as key criteria (Sampling Methods, Analytical Methods) and other criteria (data about metabolites). The risk of bias for each parameter was evaluated as "low", "medium", "high", or "not applicable". The studies for which all the key criteria and most of the other criteria are characterized as "high" were excluded.

3. Results

The flow diagram for selecting studies is shown in Figure 1. The initial database search yielded 441 studies, from which 133 abstracts were examined and 40 full texts were evaluated for eligibility. A total of 18 studies were rejected after examining the inclusion and exclusion criteria, primarily because they were related to biological samples collected from the sawmill workers. A total of 23 papers on microbial occupational exposure were chosen.

Characteristics and Data Obtained in the Selected Studies

Table 2 describes the main characteristics from the selected studies. From the selected studies (N = 23), 15 were conducted in the Europe, namely 5 in Norway [29,36–39], 4 in Poland [8,40–42], 2 in Switzerland [43,44], 2 in Croatia [45,46], 1 in Finland [47], 1 in Italy [48], and 1 in France [30]. Five studies from Canada [49–53], 1 from Korea [54], and 1 from Iran [55] were also analyzed. The majority of studies (15 out of 23–65.2%) analyzed environmental samples from small and medium size sawmills [18,28,36–49,51,52], 2 studies (8.7%) were performed in industrial sawmills [29,39], 2 studies (8.7%) in plywood hardwood processing companies [53], 1 (4.4%) in a manufacturing industry [51], 1 (4.4%) in carpentries [48], 1 (4.4%) in pellet production facilities [42], and 1 (4.4%) in a furniture factory [41].

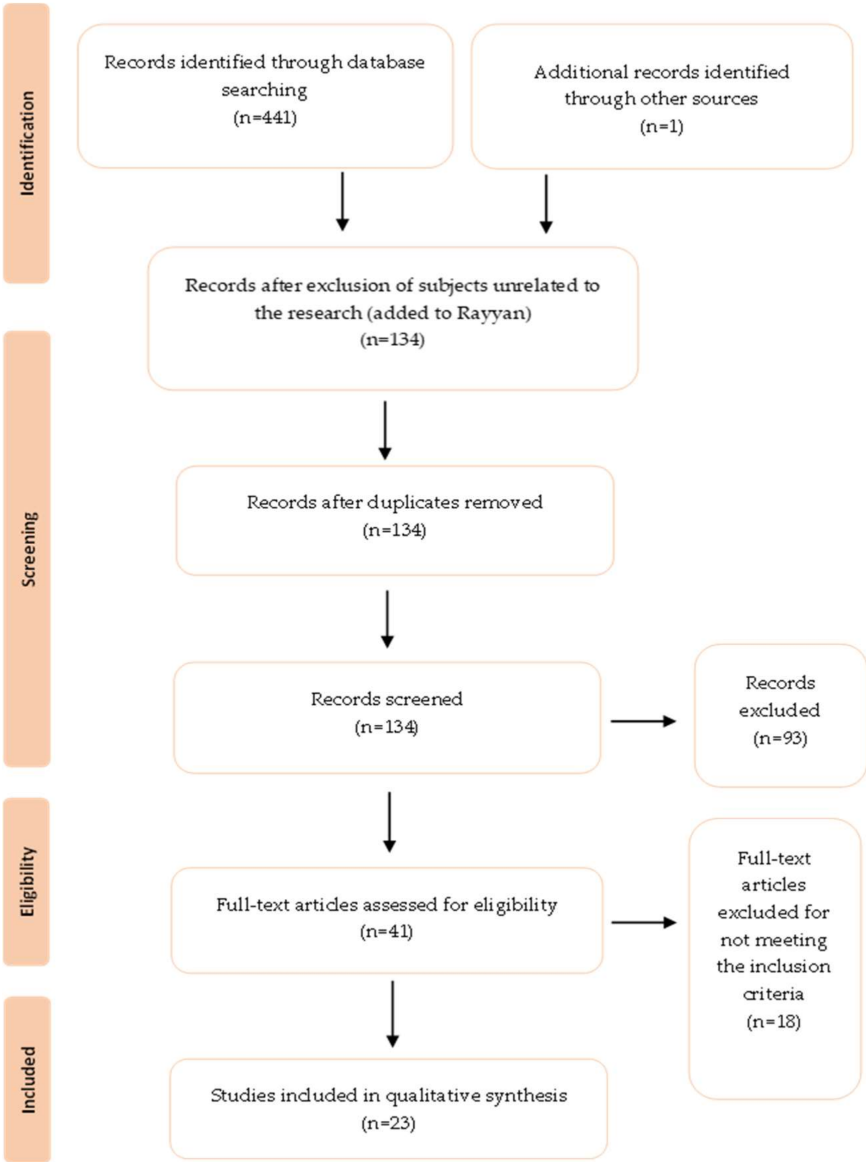

Figure 1. PRISMA based selection of articles.

Table 2. Data selected from the chosen papers.

Title	Occupational Environments	Sampling Methods	Analytical Methods	Main Findings Concerning Microbiological Contamination	Ref.
Assessment of Particulate and Bioaerosol in Eastern Canadian Sawmills	Sawmills (N = 17)	Active—Filtration, Impaction and Impinger	Morphol. id. (Fungi)	*Penicillium* sp. was the predominant genera, with up to 40 different species identified. The highest levels of molds, bacteria were associated to debarking site. Planing sites were the most highly dust contaminated. Airborne biological contaminants vary between working sites and their microflora diverge from that previously described in European sawmills.	[49]

Table 2. *Cont.*

Title	Occupational Environments	Sampling Methods	Analytical Methods	Main Findings Concerning Microbiological Contamination	Ref.
Airborne microfungi from eastern Canadian sawmills	Sawmills (N = 17)	Active—Impaction and Impinger	Morphol. id. (Fungi)	In eastern Canadian sawmills, the micoflora is dominated by *Penicillium* species. Fungi identified in European sawmills were not frequently identified in eastern Canadian sawmills.	[50]
Assessment of Bioaerosols and Inhalable Dust Exposure in Swiss Sawmills	Small (N = 8) and medium size Sawmills (N = 2)	Active—Filtration, Impaction and Impinger	Morphol. id. (Fungi)	All sawmills exceeded the Swiss occupational exposure guideline of 1000 Colony Forming Units CFU. m^3. Two sawmills for total bacteria and one sawmill for Gram-negative bacteria did not comply with Swiss occupational exposure guideline. Gram-positive bacteria, mainly *Bacillus* spp. were prevalent among the plates. The most frequent fungal genera was *Penicillium* sp.	[43]
Effects of biaerosol exposure on work-related symptoms among Swiss sawmills workers	Sawmills (N = 12)	Active—Impaction	Morphol. id. (Fungi)	The composition of airborne fungi exceeded the limit recommended by the Swiss National Insurance. Fungal level influenced the occurrence of bronchial syndrome. Airborne fungi in the sawmill environment are potential agents for occupational health effects.	[44]
Microbial Exposure Assessment in Sawmill, Livestock Feed Industry, and Metal Working Fluids Handling Industry	Livestock feed Industry (N = 3), Metal working Fluids Hadling Industry (N = 2) and Sawmills (N = 5)	Active—Impaction	Morphol. id. (Fungi)	Airborne concentrarion of bacteria and fungi were 1.864 and 2.252 CFU·$m^{3.}$ The ratio I/O was 3.7 and 4.1 for bacteria and fungi respectively. The respiratory fraction of bacteria was 57.7%, and fungi was 83.7%. Bioaerosol density was the highest in sawmills.	[54]
Occupational Health and Safety Issues in Ontario Sawmills and Veneer/Plywood Plants: A Pilot Study	Sawmill (N = 8) and venner/plywood manufacturing industry (N = 12)	Active—Impaction	Morphol. id. (Fungi)	Fungal bioaerosols vary between different indoor locations.	[51]
Respiratory Health and breath condensate acidity in sawmill workers	Sawmills (N = 2)	Active—Impaction and filtration	Morphol. id. (Fungi)	Airborne dust concentrations were below the threshold limit value. Airborne moulds were at levels able to induce inflammatory response in the airways. Significant differences between sawmills were observed regarding mould levels.	[45]

Table 2. *Cont.*

Title	Occupational Environments	Sampling Methods	Analytical Methods	Main Findings Concerning Microbiological Contamination	Ref.
Occupational exposure to airborne fungi in two Croatian sawmills and atopy in exposed workers	Sawmill (N = 2)	Active—Impaction	Morphol. id. (Fungi)	Airborne fungi present health hazardous levels (above 10^4 m^{-3}) in one sawmill. Fungal levels were related to saw working sites. The prevalent fungal genera were *Penicillium* (50–100%), *Paecilomyces* (43–100%) and *Chrysonilia* (33–100%). Other airborne fungi that were recurrent, but with lower frequency were: *A. niger* (15–71%), *Trichoderma* sp. (8–40%), *Rhizopus* sp. (8–20%) and *A. flavus* (2–15%).	[46]
Fungal fragments and fungal aerossol composition in Sawmills	Sawmills (N = 2)	Active—Filtration	GM (Fungal fragments); FESEM (Fungi)	The composition of fungal aerosols comprised in average: submicronic fragments (9%), large fragments (62%) and spores (29%). The ratio of spores was higher in saw departments. Fungal fragments were most prevalent in sorting and green timber departments. The season influenced significatively the fungal aerosol density but not the composition. Fungal fragments should be included in exposure-response studies.	[36]
Exposure to Wood dust, Microbial Components, and Terpenes in the Norwegian Sawmill Industry	Sawmills (N = 11)	Active—Filtration	GM (Fungal fragments FESEM (Fungi)	The GM of both thoracic and inhalabe expoure was higher in various departments. The mean fungal spore was 0.41 × 10^5 spores·m^{-3}. Exposure to spores was high in dry timber departments. High levels of thoracic fungal spores was also found in workers associated to sorting of dry timber. Microbial exposure had the highest levels in workers working with green timber.	[37]
Algorithm to assess the presence of *Aspergillus fumigatus* resistant strains: The case of Norwegian sawmills	Industrial sawmills (N = 11)	Active—Filtration	Morphol. id.; Screening—EUCAST method; Mol. tools—DNA sequencing (Fungi)	Fungal contamination ranged from 0–2.7 × 10^5 CFU·m^{-3} in malt extract agar (MEA) and from 0–1.3 × 10^5 CFU·m^{-3} in dichloran-glycerol agar (DG18). The prevalent species were *Chrysonilia sitophila* (65.20%), *Mucor* sp. (23.86%) and *Rhizopus* sp. (10.75%) on MEA. On DG18, *Penicillium* sp. (0.26%) and *Aspergillus* sp. (0.14%) were frequent. In MEA, section *Fumigati* was found. Whereas in DG18, four different *Aspergillus* sections were detected: *Circumdati; Candidi; Fumigati; Nigri*. Two *Fumigati* isolates were able to grow in the presence of one or two medical triazoles. One isolate was found to be a TR34/L98H mutant. Fungicides used at sawmills may decrease fungal sensibility to azole drug	[29]

Table 2. *Cont.*

Title	Occupational Environments	Sampling Methods	Analytical Methods	Main Findings Concerning Microbiological Contamination	Ref.
Respiratory Healh Impact of Working in Sawmills in Eastern Canada	Sawmills (N = 17)	Active—Impaction and Impinger	Morphol. id. (Fungi)	The most frequently fungal identified were *Penicillium myczinskii*, *P. spinulosum*, *P.fellutanum*, *Trichoderma* sp. and *Paecilomyces* sp. Working in a Québec sawmill does not constitute a clinically revelant respiratory Health risk.	[52]
Gram-negative bacteria associated with timber as a potential respiratory hazard for woodworkers	Sawmills (N = 1)	Active—Impaction Passive—Wood samples	Biochem. tests (Bacteria)	*Enterobacteriaceae* strains, by majority *Enterobacter* sp. and *Rahnella* sp. comprised 70–75% of Gram-negative bacteria isolates from pine and beech wood and sawmill air samples. During processing of beech wood high levels of Gram-negative bacteria were released into air, when comparing with pine wood processing. The aerial exposure to Gram-negative bacteria possessing endotoxic and allergenic properties poses a potential risk to workers health.	[40]
Functional disorders of the lung and symptoms of respiratory disease associated with occupational inhalation exposure to wood dust in Iran	Sawmills (N = 20)	Active—Impaction and filtration	Morphol. id. (Fungi); Biochem. tests (Bacteria)	The prevalent Gram-negative bacteria were Pseudomonadaceae, *Klebsiella pneumoniae* and *Rhinoscleromatis* sp. *Penicillium* sp. and *Fusarium* sp. were the predominant fungi. Respiratory symptoms were significantly more frequent among exposed workers.	[55]
Exposure Determinants of Wood Dust, Microbial Components, Resin Acids and Terpenes in the Saw- and Planer Mill Industry	Sawmills (N = 11)	Active -Filtration	FESEM (Fungi)	The highest microbial exposure were estimated in the green part of the sawmills. Exposure to fungal spores were relatively low and similar among most departments. Season and wood type had a large effect on the estimated exposure.	[38]
The Inhalable Mycobiome of Sawmill Workers: Exposure Characterization and Diversity	Industrial sawmill, sorting mill and planer mill companies processing spruce or pine (N = 11)	Active -Filtration	FESEM (Fungi); GM (Fungal fragments); Mol. tools (DNA-sequencing) (Fungi)	*Ascomycota* was the common phylum detected (50.3%) followed by *Basidiomycota* (45.6%). Operational taxonomic units were higher during spure processing when compared to pine processing. The highest fungal diversity was obtained in saw department. The fungal compositions of the exposures differs between seasons, sawmills, wood types and departments. A risk assessment based on the fungal diversity diferences should be performed.	[39]

Table 2. *Cont.*

Title	Occupational Environments	Sampling Methods	Analytical Methods	Main Findings Concerning Microbiological Contamination	Ref.
Exposure to airborne microorganisms in polish sawmills	Sawmills (N = 4)	Active—Impaction	GM (Fungal fragments) Morphol. id. (Fungi); Biochem. tests (Bacteria)	Microorganisms load was higher in sawmills processing coniferous wood when compared to those processing deciduous wood. Allergenic fungi (the majority *Aspergillus fumigatus*) were predominant in air samples when debarking. During first-cut frame airborne microflora as mostly constituted by endotoxin producing Gram-negative bacteria belonging to *Rahnella* genus developing in the sapwood of pine. Regarding bacteria diversity, 34 species or genera were identified. Also, 21 species or genera of fungi were found in the air of sawmills. Workers of Polish sawmills may be exposed during some tasks to airborne microorganisms posing respiratory hazard.	[8]
Fungal Spores As Such Do Not Cause Nasal Inflammation In Mold Exposure	Sawmill (N = 11)	Active—Impinger (personal samplers)	Epifluorescence technique CAMNEA method (Fungal spores)	*Rhizopus* and *Penicillium* were the predominant genera. Proinflammatory potential of microbial exposure seems to be related to the type of microbial bioaerosols in the occupational environment.	[47]
Airborne Microorganisms, Endotoxin and Dust Concentration in Wood Factories in Italy	6 Sawmills and carpentries (N = 6)	Active—Impaction and filtration	Morphol. id. (Fungi); Biochem. tests (Bacteria)	In air samples from wood factories 19 species of Gram-negative and 14 species of Gram-positive bacteria were identified. Whereas, 18 species of mould were found, some having allergenic, immunotoxic properties. Gram-negative bacteria levels were higher in these workplaces. *Penicillium* sp. and *Alternaria alternata* were identified in low densities. Workers in wood factories may be exposed to high levels of inhalable dust.	[48]
The evaluation of microfungal contamination of dust Created during woodworking in furniture factories	Furniture factories (N = 3)	Passive—settled dust	Morphol. id. (Fungi)	The most frequent fungi in the tested dust were *Penicillium* sp. and *Aspergillus* sp. *Trichoderma* genus has been isolated.Airborne fungal may be associated with the wood dust, posing a health hazard for exposed workers.	[41]
Hypersensitivity Pneumonitis in a Hardwood Processing Plant Related to Heavy Mold Exposure	Hardwood processing plant (N = 1)	Active—Impaction Passive—Dust and surface samples from wood planks	Morphol. id. (Fungi)	*Paecilomyces* sp. growth was observed on the surface of the dried processed wood in the index plant. *Penicillium* sp. was prevalent on green wood. Wood quality (moisture content, time of storage prior to drying) and processes may influence wood contamination workers exposure.	[53]

Table 2. *Cont.*

Title	Occupational Environments	Sampling Methods	Analytical Methods	Main Findings Concerning Microbiological Contamination	Ref.
Nasal lavage and analytical tool in Assessment of exposure to particulate and microbial aerossol in wood pellet production facilities	10 Pellet production facilities (N = 10)	Active—Impaction and filtration	Morphol. id (Fungi and Bacteria); Mol. tools (DNA sequencing) (Fungi and Bacteria) Biochem. tests (Bacteria)	Among isolated, bacterial pathogens from *Streptomyces* genus and *Aspergillus fumigatus* pathogenic fungus were identified. Concerning microorganisms size distribution, the highest bacteria load can reach the nasal and oral cavities as well as secondary bronchi. In case of fungi, the highest load can reach the nasal and oral cavities. Microbiota diversity in the indoor was higher when compared to the outdoor, suggesting that the processed material act as an active emission source.	[42]
Azole-resistant *Aspergillus fumigatus* in sawmills in Eastern France	Sawmills (N = 20)	Active—Impaction Passive—Settled dust	Morphol. id. Screening (EUCAST and E-test); Mol. tools (DNA-Seq) (Fungi)	Azole resistante *A. fumigatus* was collected in 20 samples from a total of 600 settled dust samples. From the *A.fumigatus* obtained strains, 83% had TR34/L98H mutation. A greater number of resistant strains was collected in sawmills that applied fungicide products. Azole-resistant mutations seems to be associated to the azole fungicide formulation and quantities of azole.	[28]

Morphol. id.—Morphological identification; Mol. tools—Molecular tools; Biochem. tests—Biochemical tests; GM—Gravimetric Measurement; FESEM—Immunolabeling method for field emission scanning electron microscope.

The most used sampling method was air sampling (19 out of 23–82.6%) [18,29,36–40,43,44,46,47,49–55]. Several studies used more than one active sampling method (8 out of 23–34.8%). Air collection through impaction was used in 16 studies (69.6%) [8,40,42–46,49,50,52–55], followed by filter air sampling in 11 studies (47.8%) [28,29,37,38,41,44,46,48,50,53,54], while 5 studies (21.7%) used the impingement method [29,47,49,50,52].

Passive methods were exclusively performed in 5 papers (21.7%) [28,40,41,47,53]. Dust samples collection was the most frequent methodology applied (N = 3) [28,41,53], one study collected wood samples [40] and the other performed surface samples [53].

Concerning analytical procedures for microbial characterization, 13 studies (56.5%) referred to fungi [28,29,37–39,41,45–48,50,51,53], 1 (4.4%) referred only to bacteria [40], while 9 (39.1%) encompassed fungi and bacteria [8,42–44,49,50,52,54,55]. Morphological identification was the most frequent approach. Fungal identification was accomplished through macroscopic and microscopic examination in 16 studies (69.6%) [8,28,29,41–46,49–55]. Regarding bacterial identification, 5 studies (21.7%) used biochemical tests [8,40,42,50,55].

Screening for fungal susceptibility to azoles was performed in 2 studies (8.7%). For the screening of A. fumigatus azole resistance, 1 study (4.4%) used the EUCAST methods [53] and the other used both EUCAST and E-test methods [28].

Molecular tools were applied in 4 studies (17.4%). All performed DNA sequencing [28,29,39,42,55]. High fungal levels were frequent in 6 studies (26.1%) [8,44–46,50,54], as well as high bacteria levels in 4 studies (17.4%) [8,43,50,54]. Fungal identification evidence Penicillium as the most frequent genera [41,43,46,47,49,50,52,53,55]. Aspergillus sp. was also recurrent in 4 studies (17.4%) [8,29,42,46]. From all the sampling sites, 3 studies (13%) reported the sorting and green department as having the highest levels of fungal fragments [36–38]. Other working sites were also associated with potential microbial exposure as follows: saw departments [36,39], dry timber departments [37], and debarking site [49]. In fact, 7 studies (30.4%) report airborne fungi as potential agents for occupational health effects [8,41,42,44–46,50], as well as bacteria in 2 studies (8.7%) [40,56]. In what concerns mycological diversity, 3 studies (13%) report fungal bioaerosols variation between different indoor locations [39,49,51] and 4 studies (17.4%) evidence a significant influence of seasons in fungal aerosol composition [36,38,39,49].

4. Discussion

It is well known that sawmill workers are exposed to wood dust and multiple wood-associated chemicals and microbiota, including fungi [1–4,30,31]. Fungi and Gram-negative bacteria are major contaminants of wood dust, especially in hot and humid areas. Occupational inhalation exposure to wood dust and its associated bioaerosols (composed by fungi, bacteria, endotoxins, mycotoxins, and much more) has been associated with adverse respiratory effects [5–9]. Health outcomes associated with the inhalation of wood dust have been reported in several studies [5,9,11–13,15–20] as well as a significant association between inhalation of wood dust and an increased prevalence of respiratory symptoms [13,21–23,28] and decreased lung functional capacity [55]. Considering the papers included in this review, most of them (21 out of 23) used air as an environmental matrix, impaction being the most frequent sampling method used (15 out of 23). This sampling approach relies solely on culture-based methods, which can have advantages and disadvantages. The inflammatory and/or cytotoxic potential can affect the microorganism viability [56,57] which makes this method beneficial since it allows us to rely on the microbial composition to draw conclusions regarding the inflammatory potential variation [57,58]. In impaction sampling devices, a specific flow rate (depending on the type of environment) is defined to collect particles [59] by using its inertia to drive deposition on a collection media by promoting particle separation through an air stream [60]. However, since it only allows to evaluate culturable microorganisms, the microbial load can be underestimated, due to the high velocity of the air flow that may result in microorganisms' cell damage [61,62]. Moreover, it is important to highlight that indoor air is not homogeneous in space or time, it can always change depending on the type and intensity of the activity developed in that space [63]. Therefore, the sampling time must be adequate to the environment in study and work tasks being developed. For example when using high volume samplers in highly contaminated areas, it is crucial to employ short sampling intervals and lower flow rates for airborne fungal sampling [64]. Nevertheless, active sampling methods, namely impaction devices, have already proved to be very useful in the characterization of occupational exposure to fungi in several studies, by presenting the most diversified fungal contamination in comparison with all sampling methods applied [28,51,61,65,66].

Passive sampling methods were also used, even if in a smaller number (3 out of 23 papers, including studies with one or more sampling methods). There is evidence that ventilation, building design, environmental features [67], or water infiltrations and damage [68], geographical location [69], as well as the type of task developed in each working site [36,49] can alter fungi and bacteria found indoors. Different working sites were identified with potential for microbial exposure namely the ones that include sawing and drying, mainly because the cells in hardwood are firmly bonded, and kiln drying renders them less elastic, resulting in cell breakage and tiny airborne dust [70,71].

With so many factors impacting microbial contamination indoors, passive sampling approaches are anticipated to be more reliable than active sampling methods since they can collect contamination over a longer period of time, thus covering all expected fluctuations [72,73]. The passive sampling method used in all three studies was the collection of wood dust, which both acute and chronic exposures may serve as a sensitizer and irritant on the human body, mostly affecting the respiratory system and skin [56].

Several researchers [67,73–78] have begun to collect and analyze from indoor environments a similar matrix (settled dust) as part of their microbial contamination exposure assessments. Settled dust reservoirs have been described as having the ability to anticipate microbial levels in indoor air, as well as being more repeatable than active sampling approaches [67]. Furthermore, it has been documented as an environmental support for bacterial development, and is thus regarded as a bacterial contamination reservoir [79].

Considering all the described advantages and disadvantages of both active and passive sampling methods and in order to assess microbial exposure, sampling approaches in occupational environments should comprise more than one type of sampling method [28,29,62,67,73,76]. Furthermore, and as it was seen in one study, settled dust should be included in sampling protocols combined with impaction methods because when these two methods are combined, the sensitivity of the assessment increases, and the impaction samplers' shortcomings are eliminated [58,80].

The majority of articles (15 out of 23) relied solely on culture-based methods to perform microorganisms' identification; nevertheless, and as expected, this assay also has its drawbacks that may influence the studies accuracy, such as the specificities of each species (growth rates and requirements), that can affect the other species in a mixed culture. A very common example regarding growth rate, is the overgrowth of some species that limit the growth of other species due to chemical competition [74].

Molecular tools are well known for their features of precision, high analytical sensitivity of detection, speed, and the ability to detect and identify dead or dormant microorganisms, as well as toxigenic strains from microorganisms [58,74,80–82]. However, culture-based methods should be used every time that the exposure route is mainly happening by inhalation, due to the reasons addressed before [56,57]. Thus, culture-based methods and molecular tools should be used side by side as it was seen in a few studies (4 out of 23) of this review.

Regarding the contamination present in all studies, as previously mentioned, majority of studies reported airborne fungi as a potential agent for occupational health effects (10 out of 23) since the prevalent genera were *Penicillium* (9 out 23) and *Aspergillus* (4 out of 23). *Aspergillus* sp. can be found everywhere and are easily disseminated in the air. Because the conidia of the *Aspergillus* genus are so small, they can readily be inhaled and colonize the upper and lower respiratory tracts of those who have been exposed [83,84]. Therefore, and as a consequence of a high exposure to opportunistic *Aspergillus* sp. (both in clinical and environment) the number of infections in immunocompromised patients has increased, as well as the antifungal resistance. It is known that *Aspergillus* species with a pathogenic potential, such as *A. flavus, A. niger, A. terreus, A. versicolor, A. calidoustus*, and *A. nidulans* [29,85], can lead to several health outcomes such as allergic bronchopulmonary aspergillosis and chronic pulmonary aspergillosis [58,86]. Additionally, it is also crucial to evaluate those species resistance to azoles, as it was performed in two studies of this review, in which the authors made a screening for *A. fumigatus* susceptibility to azoles. Azole resistance is a growing issue in *A. fumigatus*, threatening clinical improvements made possible by the use of azole antifungals in the treatment of *Aspergillus*-related disorders [28]. While some fungal species have innate azole resistance, acquired azole resistance has been found in fungi from occupational environments, such as sawmills, where azole fungicides (14-alpha demethylase inhibitors, DMI) used for timber preservation may exert some selection pressure on fungal populations [29]. Therefore, the use of azole fungicides to protect the wood reinforces the idea of performing a screening of susceptibility to azoles, specifically in this occupational environment.

Despite the methods used for the microbial occupational exposure assessment in these studies, it is important to highlight other methods and analysis that allowed a more complete assessment of sawmills' workers occupational exposure, such as the assessment to fungal allergens [87]. Sawmill workers are exposed to large levels of allergenic fungus on a regular basis, which can cause respiratory problems and asthma [8,87,88]. Microscopical spore counts and culture-based approaches have historically been used to measure fungus exposure [89]. There are, however, various immunoassays to measure environmental antigens [90] like the enzyme-linked immunosorbent test (ELISA) [87]. Another method commonly used in the studies of this review (9 out of 23) was the limulus amoebocyte lysate assay (LAL) to analyze and quantify endotoxins, and the field emission scanning electron microscopy (FESEM) to analyze fungal particles.

It is important to highlight that none of the studies included mycotoxins assessment. Mycotoxins are secondary metabolites created by fungi, and together with endotoxins and glucans, they make products of fungi and bacteria that are present in the organic dust produced by organic materials, including soil, plants, animals, food, and faeces, and inhaled by workers in a variety of industries [91]. Some mycotoxins can have serious human health effects when ingested, but their health effects following inhalation or dermal contact are insufficiently documented [91].

Specific fungal genera, primarily *Aspergillus, Penicillium, Alternaria, Fusarium*, and *Claviceps*, produce mycotoxins [91–93], such as aflatoxin B1 (produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*), ochratoxin (produced by both *Aspergillus* and *Penicillium*), trichothecenes, zearalenone, fumonisins B1 and B2, and some emerging mycotoxins like fusaproliferin, moniliformin, beauvericin and enniatins (produced mainly by *Fusarium* species), ergot alkaloids, (produced by *Claviceps*) and altenuene, alternariol, alternariol methyl ether, altertoxin, and tenuazonic acid (produced by *Alternaria* species) [91,93–95]. Two of them (*Penicillium* and *Aspergillus*) were found with the highest prevalence in this setting.

Mycotoxins can exist in the environment even when no visible fungi are present [91,96], since they can withstand adverse environmental factors such as high or low temperatures and can persist long after the death and disintegration of the fungal species responsible for their production. Even after being exposed to temperatures such as boiling or roasting operations, they are difficult to eradicate or inactivate from the source [91,97]. The majority of mycotoxins are non-volatile, nevertheless, they can be found in airborne dust [88,92,93], as well as in fungal spores and fragments [91,96,97]. As a result, dust, spores, and hyphae fragments in the air can carry mycotoxins to the lungs [91,96,97]. Moreover, in other cases, exposure in the workplace happens primarily by inhalation, notably through airborne dust [88,93–95,98–102]. Mucous membrane irritation, skin rash, nausea, immune system suppression, acute or chronic liver damage, acute or chronic central nervous system damage, endocrine changes, and cancer are all signs and effects of inhaling mycotoxins [91,97,103–105].

As previously reported by Viegas and colleagues [91], although the health effects of exposure to some mycotoxins through eating of contaminated food are well documented, few research has looked into the health implications of mycotoxins through inhalation or skin contact and absorption, which are probably the main routes of exposure in the sawmills industry. To understand the main determinants that may have an impact on exposure, it is particularly important to properly characterize occupational exposure through the identification of current mycotoxins, their levels, duration, and main routes of exposure associated with specific occupational environments. In addition, to allow comparisons between research standardized techniques (sampling and analysis) are required [91].

Finally, the geographical distribution of the studies included in this review is also something to consider since most of them (15 out of 23) were conducted in Europe. Thus, it is evident that there is a lack of investigation regarding microbial exposure in this occupational environment in the rest of the world. Moreover, looking more closely at the distribution of studies in Europe, the imbalance in the various areas is also perceptible since most studies are from Northern Europe (6 out of 15) and Central Europe (8 out of 15), leaving areas like Western Europe and Southern Europe with one study each, and Eastern Europe without studies regarding this subject.

Combining the findings of this review with the lack of information, it is possible to highlight the need to increase investigation regarding microbial occupational exposure in sawmills all over the world. This paper's findings should be considered, when preparing sampling campaigns and laboratory resources, to achieve an accurate microbial occupational exposure assessment in Portuguese sawmills.

5. Conclusions

This review allowed to identify the sampling methods and assays already employed to assess occupational exposure to microbial contamination in sawmills and to identify the knowledge gaps in what concerns this risk characterization.

Sawmill workers are exposed to several microbial contaminants in their workplace. Exposure to bacteria and fungi has been already reported, as well as bacteria metabolites (namely endotoxins). However, mycotoxins' assessment was not yet performed and, therefore, the risk from this exposure was not estimated.

No papers were found reporting the occupational microbiological exposure in sawmills located in Portugal. Therefore, microbial occupational exposure assessment in Portuguese sawmills is crucial to better characterize this risk, and to identify the measures to be taken into account in order to protect the workers.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/atmos13020266/s1, Table S1. PRISMA Checklist.

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