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ORIGINAL PAPER

Occurrence and characterization of culturable bacteria and fungi in metalworking environments

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Abstract Modern metalworking fluids (MWFs) provide the breeding ground for microorganisms that could enter the ambient environment via aerosolization. This manuscript describes the occurrence, type, concentration and size distribution of bacteria and fungi in an MWF environment by sampling aerosols and MWF sumps. In this study, computer numerical control (CNC) thread-cutting and grinding processes at a socket-manufacturing plant were investigated. *Cladosporium* and *Aspergillus* were the dominant fungal isolates in this study. Peak fungal concentrations (797 CFU/m^3) were observed during thread-cutting processes, and the most prevalent fungi were in the size range of 2.1–3.3 μm. Moreover, *Exiguobacterium* spp., *Micrococcus* spp., and *Staphylococcus capitis* were the dominant bacterial isolates identified. The most prevalent bacteria were in 4.7–7.0 μm size range. This study indicates that the type of machine tools was not associated with MWF sump microbial concentrations. The total bacterial and fungal contamination in MWF sump samples reached levels of 10^5 CFU/ml. *Pseudomonas* spp. was the only bacterium identified in the MWF sumps, and *Aspergillus*, *Penicillium,* and yeast were the predominate fungi identified.

Keywords Metalworking fluids · Bioaerosols · Bacterial · Fungi · Thread cutting · Grinding

1 Introduction

Metalworking fluids (MWFs) are pure mineral oils or water-based fluids that are poured or sprayed onto metals to lubricate, cool and prevent corrosion as well as to remove metal scraps during machining operations. Water-based MWFs, especially, are used in industrial environments, and the use of these MWFs during mechanical operations often results in the formation of aerosols that are associated with adverse health effects. These effects can include respiratory illnesses such as hypersensitivity pneumonitis (HP) (Hodgson et al. 2001) as well as rectal cancer (Malloy et al. 2007), rhinitis-related symptoms (Park et al. 2008), and dermatitis (Ueno et al. 2002).

Mycobacterium and *Pseudomonas* are two important genera that have been quantified by agar plating

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in contaminated MWF and have been implicated in MWF-linked occupational illnesses (Wallace et al. 2002). The growth of acid-fast bacilli of the genus *Mycobacterium* in these fluids has provided evidence for a link between *Mycobacteria* and HP in machine workers exposed to MWFs (Bernstein et al. 1995; Muilenberg et al. 1993; Kreiss and Cox-Ganser 1997). *Pseudomonas* species and their endotoxins also have been linked to HP in machine workers (Bernstein et al. 1995). Based on these linkages, assaying the species distributions of bioaerosols is imperative to assess their potential implications for respiratory illness.

There are no generally accepted guidelines or standards for total fungal and total bacterial bioaerosols. The American Conference of Governmental Industrial Hygienists (ACGIH) Bioaerosol Committee has yet to give a numerical threshold limit value (TLV) for fungal bioaerosols, most likely due to a lack of consensus and scientific evidence. The ACGIH committee has also indicated that total bacterial level in excess of 500 CFU/m^3 in workspaces of affected individuals is unsafe (ACGIH 1986). The guideline set by the World Health Organization for the bioaerosol counts of total bacteria is 500 CFU/m^3 (World Health Organization, WHO 1990; WHO 2002).

To date, bioaerosol exposure among workers using MWFs in several different industries has been assessed. Targeted groups include automobile-manufacturing workers (Malloy et al. 2007), automotive ring-manufacturing workers (Park et al. 2008), and machine operators (Lillienberg et al. 2008).

Socket manufacturing is a vital industry for the success of many other companies and industries, and there is too great a consumption (by inhalation) of metalworking fluids in these environments (Park et al. 2009). Modern metalworking fluids provide a breeding ground for microorganisms that could enter the ambient environment via aerosolization. However, to the best of our knowledge, the biohazards associated with concentration, species, and size distribution of airborne culturable fungi and bacteria found MWFs have not been well characterized in socket-manufacturing industries. Therefore, the aims of this survey were not only to evaluate the levels of microorganisms in the metalworking environment but also to investigate the effects of the metalworking procedures of thread cutting and grinding on airborne bioaerosol distribution.

2 Materials and methods

2.1 Air sampling

The investigations were performed at a socket-manufacturing plant located in Taiping City, Taichung, Taiwan. This plant, established 25 years ago, employs 60 workers. The manufacturing of sockets involves several important processes, including surface treatment, cutting, grinding, electroplating, cleaning, packaging and shipping. All the sites that use MWFs in their operations were chosen as field sites for this study. Air samples were taken at the following sites: (1) the computer numerical control (CNC) thread-cutting machines ($n = 6$), (2) grinding machines ($n = 6$), (3) the staff office and (4) outdoors. Air samples collected from the staff office were used as the control. Sampling trials were carried out from April to July 2009. In all cases, samples were collected at a height that coincided with the workers' breathing zone. Parallel outdoor and indoor air samples were taken simultaneously. First, a pilot study of the sampling times for fungi and bacteria was conducted in all the sampling sites. Each air sample was collected over a 3-min period because this length of time appeared to adequately represent the overall pattern of exposure concentrations. Triplicate samples were taken at each sampling site, providing a total of 54, 36, 9 and 9 samples, for thread-cutting sites, grinding sites, outdoor, and office, respectively.

Meteorological factors, including temperature (°C) and relative humidity (RH) (%), were recorded by a thermo hygrometer (SHINYEI CTH-888, Osaka, Japan) during each sampling event. Airborne bioaerosols were collected with the one-stage microbiological air sampler (MAS-100), consisting of a plate containing 400 holes 0.74 μm in diameter (Merck, Dermstadt, Germany). Total culturable bacteria and fungi concentrations between the MAS-100 and Anderson 1-stage impactor are quite close (Lee and Jo 2006). The MAS-100 air sampler was selected for this study because there are high levels of bioaerosols in environments with metalworking fluids and it has been reported to have excellent capturing performance, low cost, and simplicity of use (Nesa et al. 2001). Air samples were collected at a flow rate of 100.0 l/min and impacted on 90 mm Petri dishes containing malt extract agar (MEA) or trypticase soy

agar (TSA) for sampling fungi and total culturable bacteria, respectively.

To investigate the size distribution of the bioaerosols at different sites, a six-stage Andersen impactor (Andersen Sampler Inc. Atlanta, CA) was employed for sampling at each of the three sites. The Andersen 6-stage sampler was a cascade impactor with 400 holes per stage, drawing in air at a flow rate of 28.3 l/min. The device has particle cutoff sizes of 7.4, 4.7, 3.3, 2.1, 1.1, and 0.65 μm for stages 1 through 6, respectively. The cutpoints for the Anderson impactors are based upon calculated D_{50} and that in fact, there is a wider range of spore size that could have been collected on any given impaction plate. Therefore, particles larger than the calculated D_{50} on an impaction stage are collected with greater efficiency. Triplicate samples were taken at each sampling site, providing a total of 324, 216, and 54 samples, for thread-cutting sites, grinding sites, and outdoor, respectively.

2.2 Bioaerosol analysis

The fungal (MEA) and bacterial plates (TSA) were incubated at 25°C for 5 days and 37°C for 2 days, respectively. Plates that were either contaminated or had produced considerable overgrowth were excluded. The resultant colonies were counted using visual inspection and converted to an effective count by the positive-hole correction table (Feller 1950). Colonies growing on both media were enumerated and calculated as colony-forming units per cubic meter (CFU/m^3). Fungal isolates were identified according to colony appearance as well as spore and morphological characteristics (de Hoog et al. 2000; Larone 2002). Bacterial isolates were Gram stained, and bacterial colonies were identified on the basis of colony characteristics and the morphological characteristics of spores, as recommended by Bergey's Manual. The selected bacterial isolates were identified using microtests such as the API (Analytical Profile Index) system 50 CHB, STAPH, 20E, 20NE, and CORYNE. The isolation frequency ("relative frequency") of fungi or bacteria was defined as the percentage of isolates of individual species in relation to the total number of isolates. In addition, the bacterial genus identification was confirmed by sequence analysis of 16S ribosomal DNA, which was conducted by Mission Biotech Crop., Taipei, Taiwan. All sequence results were compared with the sequences of the NCBI cDNA library, and the resulting similarities were more than 99%.

The analysis method of bioaerosol in MWF sumps is based on the previous method (Veillette et al. 2004). This study was conducted in a socket-manufacturing plant in which water-based MWFs were used. All samples of MWF sumps were taken from a flowing stream at each cutting or grinding machine when the circulation system was in operation. If the system was not in operation, the MWF circulation system was run for at least 10 min prior to sampling, according to Bennett's recommendation (1972). These samples were collected in a 250-ml sterile bottle. Then, the MWFs were cultured for bacteria and fungi. The total bacterial and fungal counts reflect MWF sump/fluid counts.

2.3 Statistical analysis

Statistical analyses were carried out using the SPSS statistical software package (Version 12.0 for Windows). Non-parametric Mann–Whitney tests and Kruskal–Wallis tests were applied to compare data sets. The level of significance was set at $P < 0.05$ (two tailed).

3 Results

3.1 Identification and concentration of airborne bacteria at different sampling sites

In this study, the mean outdoor temperature was 33.1–33.5°C, and the mean temperature in the office was 28.6–30.6°C. Similar mean temperatures (32.4 and 32.1°C) occurred at the thread-cutting and grinding sites, respectively. The average indoor RH was 50.0–70.4%, and the outdoor average RH was 65.7–68.3%. The lowest RH (50.0%) occurred at the office site, and the highest RH (70.4%) occurred at thread-cutting sites. One of the main reasons for this phenomenon was a large amount of water-based MWF use at thread-cutting sites.

Based on this study, the total number of bioaerosol measured for most indoor environments was below 1,000 CFU/m^3. Additionally, the mean bacterial concentrations at the sampling sites were also found to be below 500 CFU/m^3, the guideline set by the

WHO. The highest airborne bacterial concentration was measured in the office, which bacterial concentration is higher than 500 CFU/m^3.

The total bacterial concentrations at thread-cutting sites were higher than at grinding sites, but with no statistical difference ($P > 0.05$). However, levels of *Micrococcus* spp. ($P = 0.035$), *Staphylococcus capitis* ($P = 0.011$), *Mycobacterium* spp. ($P = 0.019$), *Bacillus pumilus* ($P < 0.05$), *Bacillus circulans* ($P = 0.02$), and *Aeromonas hydrophila* ($P = 0.01$) were significantly different between the two groups of sites. Additionally, *Exiguobacterium* spp., *Micrococcus* spp., and *Staphylococcus capitis* were the dominant bacterial isolates identified in both thread-cutting sites and grinding sites. These isolates were found in thread-cutting sites with relative frequencies of 46.9, 15.7, and 10.1%, and with mean values of 108, 36, and 23 CFU/m^3, respectively (Table 1). The remaining bacterial isolates were *Mycobacterium* spp., *Bacillus pumilus*, *Pseudomonas* spp., *Neisseria mucosa*, *Aeromonas hydrophila*, *Bacillus circulans*, *Pseudomonas aeruginosa*, *Endophytic bacterium*, and *Staphylococcus lentus*, which varied in relative frequency from 8.6 to 0.2%. In grinding sites, on the other hand, those dominant bacterial isolates were found with relative frequencies of 29.9, 27.2, and 26.3% and with mean values of 53, 49, and 47 CFU/m^3, respectively. The remaining bacterial isolates were *Bacillus circulans*, *Neisseria mucosa*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, and *Staphylococcus xylosus*, which varied in relative frequency from 12.4 to 0.5%.

As shown in Table 1, *Micrococcus* spp., *Staphylococcus capitis,* and *Mycobacterium* spp. were the dominant bacterial isolates found in the office environment, with relative frequencies of 52.1, 26.8, and 10.5% and mean values of 231, 119, and 47 CFU/m^3, respectively. The remaining bacterial isolates were *Exiguobacterium* spp., *Pseudomonas* spp., *Bacillus circulans*, *Staphylococcus xylosus,* and *Neisseria mucosa*, which varied in relative frequency from 7.5 to 0.3%. As for the bacterial isolates from the external environment, *Micrococcus* spp., *Staphylococcus capitis,* and *Mycobacterium* spp. were the predominant species, with relative frequencies of 26.5, 24.1, and 13.3%, and mean values of 24, 22, and 12 CFU/m^3, respectively. Other bacterial isolates, in decreasing order of frequency from 10.8 to 1.2%, included *Exiguobacterium* spp., *Neisseria mucosa*, *Bacillus endophyticus*, *Pseudomonas* spp. *Bacillus circulans*, *Bacillus* spp., *Endophytic bacterium,* and *Bacillus pumilus*.

Overall, *Micrococcus* spp. and *Staphylococcus capitis* were the dominant bacterial isolates obtained

Table 1 The concentrations of culturable bacteria at four sampling sites

Species	Total mean concentration of bacteria[a] (CFU/m^3)			
	Thread cutting ($n = 54$)	Grinding ($n = 36$)	Office ($n = 9$)	Outdoor ($n = 9$)
Exiguobacterium spp.	108 (301)	53 (71)	33 (27)	10 (171)
Micrococcus spp.	36 (26)	49 (37)	231 (189)	24 (19)
Staphylococcus capitis	23 (25)	47 (48)	119 (81)	22 (27)
Mycobacerium spp.	20 (25)	0 (0)	47 (76)	12 (22)
Bacillus pumilus.	15 (28)	1 (5)	0 (0)	1 (3)
Pseudomonas spp.	12 (20)	0 (0)	8 (23)	3 (7)
Neisseria mucosa	5 (18)	3 (11)	1 (3)	7 (11)
Bacillus circulans	3 (6)	22 (63)	2 (7)	3 (7)
Pseudomonas aeruginosa	3 (14)	1 (3)	0 (0)	0 (0)
Aeromonas hydrophila	4 (10)	3 (18)	0 (0)	0 (0)
Staphylococcus xylosus	0 (0)	1(4)	1 (3)	0 (0)
Endophytic bacteria	1 (2)	0 (0)	0 (0)	1 (0)
Staphylococcus lentus	1(5)	0 (0)	0 (0)	0 (0)
Bacillus endophyticus	0 (0)	0 (0)	0 (0)	4 (10)
Bacillus spp.	0 (0)	0 (0)	0 (0)	2 (4)
Other	1(2)	0 (0)	1(3)	1(3)

[a] Arithmetic mean (AM) and standard deviation (SD)

from indoor and outdoor air samples in this study. In addition, some Gram-negative bacteria were identified in this survey. *Aeromonas hydrophila*, *Pseudomonas* spp., and *Pseudomonas aeruginosa* were the dominant Gram-negative bacteria identified in metalworking fluid environments.

3.2 Identification and concentration of airborne fungi at different sampling sites

In this study, peak fungal concentrations (797 CFU/m^3) were observed during thread-cutting processes. The culturable fungi were classified as *Cladosporium*, *Penicillium*, *Aspergillus*, *Fusarium*, *Mucor*, *Alternaria*, and non-sporulating species (Table 2). Total airborne fungi were slightly, but non-significantly, more concentrated at thread-cutting sites than at grinding sites ($P > 0.01$). However, levels of *Fusarium* ($P = 0.027$) and *Mucor* ($P = 0.011$) showed statistical difference between the two groups of sites. The dominant fungi identified at thread-cutting sites were *Cladosporium* (74.0%), *Alternaria* (6.7%), and *Aspergillus* (6.7%), with mean concentrations of 233, 21, and 21 CFU/m^3, respectively. The remaining fungi, which varied in relative frequency from 5.4 to 0.9%, were *Fusarium*, non-sporulating, yeast, *Mucor,* and *Penicillium*. At the grinding sites, the dominant fungal isolates were *Aspergillus*, *Cladosporium,* and *Mucor*, with relative frequencies of 32.9, 30.1, and 19.2% and mean values of 24, 22, and 12 CFU/m^3, respectively. The remaining fungi isolates were non-sporulating, *Fusarium*, *Penicillium,* and *Alternaria*, which varied in relative frequency from 11.0 to 1.4%.

In the office air samples, the predominant fungi identified were *Cladosporium* (41.0%), *Aspergillus* (19.7%), and non-sporulating (15.4%), with mean concentrations of 48, 23, and 18 CFU/m^3, respectively. The remaining fungi, which varied in relative frequency from 8.5 to 0.9%, were *Mucor*, *Penicillium,* and *Fusarium*.

We found that the outdoor culturable fungal concentration was very low, considering the temperature and RH. This finding agrees with other previous studies conducted in Taiwan (Lin and Li 1996; Tsai and Liu 2009). The analysis of outdoor air samples showed that *Cladosporium* (44.9%), *Aspergillus* (13.6%), and *Penicillium* (13.6%) were also the predominant fungal isolates here, with mean concentrations of 53, 16, and 16 CFU/m^3, respectively. Other fungal isolates decreased in frequency from 13.2 to 2.5% and were identified as non-sporulating, *Mucor*, *Alternaria,* and *Fusarium.* In conclusion, *Cladosporium* and *Aspergillus* were the dominant fungal isolates in both indoor and outdoor air samples.

3.3 Particle size distribution of airborne bacteria and fungi

As illustrated in Fig. 1, most bacteria at the thread-cutting sites were in the size range of 4.7–7.0 μm (22.3%), whereas the least prevalent size range was 1.1–2.1 μm (7.9%). The largest proportion of bacteria at grinding sites was in the 3.3–4.7 μm size range (21.7%), which was close to the prevalence of these bacteria at thread-cutting sites. *Exiguobacterium* spp. was the predominant bacterial isolate identified in the size range of 4.7–7.0 μm, followed by *Staphylococcus capitis* and *Pseudomonas* spp. Similarly, *Exiguobacterium* spp. was the dominant bacterial isolate

Table 2 The concentrations of culturable fungi at four sampling sites

Species	Total mean concentration of fungi[a] (CFU/m^3)			
	Thread cutting ($n = 54$)	Grinding ($n = 36$)	Office ($n = 9$)	Outdoor ($n = 9$)
Cladosporium	233 (237)	22 (30)	48 (51)	53 (83)
Aspergillus	21 (30)	24 (21)	23 (42)	16 (18)
Alternaria	21(22)	1 (4)	0 (0)	12 (18)
Fusarium	17 (18)	2 (5)	8 (13)	3 (5)
Non-*sporing*	16 (13)	8 (5)	18 (11)	14 (11)
Mucor	4 (6)	14 (16)	10 (13)	4 (7)
Penicillium	3 (5)	2 (4)	9 (17)	16 (160)
Other	0 (0)	0 (0)	1 (3)	0 (0)

[a] Arithmetic mean (AM) and standard deviation (SD)

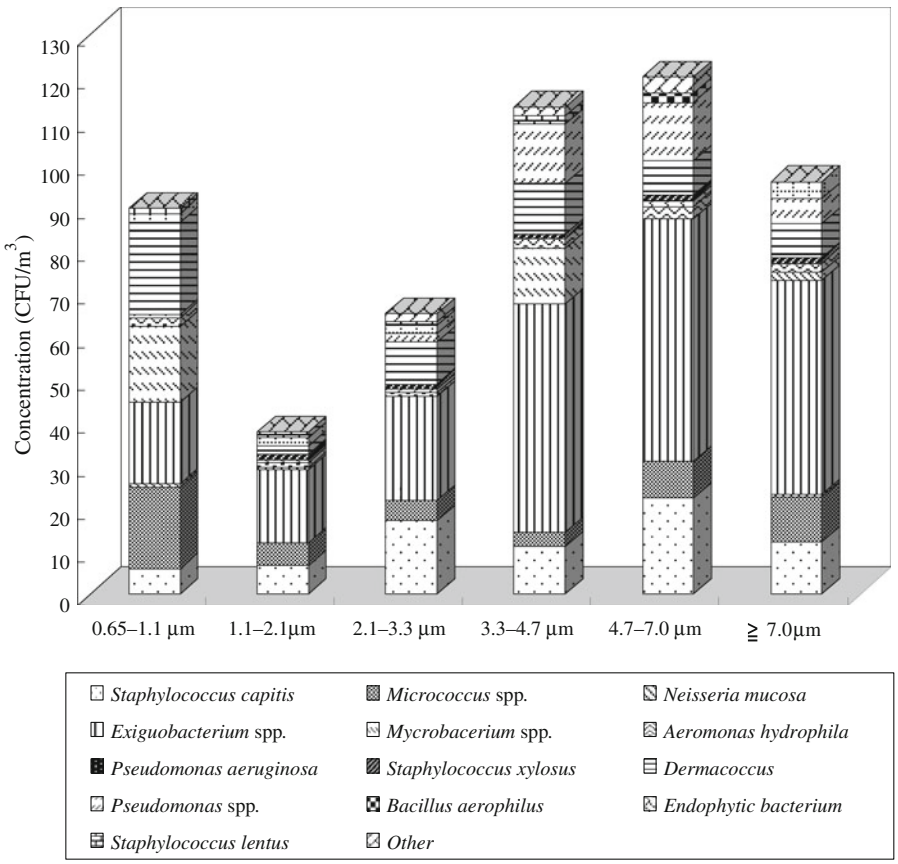

Fig. 1 The species, concentrations, and size distribution of total bacteria at the CNC thread-cutting sites

identified in the 3.3–4.7 µm size range, followed by *Staphylococcus capitis* and *Pseudomonas* spp.

Figure 2 shows that at grinding sites, the highest proportions of bacteria fell into the 3.3–4.7 µm size range (23.3%) and the lowest fell into the 2.1–3.3 µm range (8.7%). The distribution pattern of bacteria was similar to that observed at thread-cutting sites. However, the predominant bacterial isolate observed in the 3.3–4.7 µm range at grinding sites was different from that found at thread-cutting sites. *Micrococcus* spp. was the dominant bacterial isolate in the 3.3–4.7 µm size range, followed by *Staphylococcus capitis* and *Exiguobacterium* spp.

To compare the particle size distribution of airborne bacteria at thread-cutting sites and grinding sites, Mann–Whitney tests were performed. Airborne bacteria ≥7.0 µm in diameter at thread-cutting sites were more abundant than at grinding sites ($P = 0.001$). Additionally, levels of *Exiguobacterium* spp. in the size ranges of 0.65–1.1, 2.1–3.3, 3.3–4.7, and ≥7.0 µm showed statistical differences between the two sets of sites ($P < 0.05$).

Regardless of different metalworking procedures, the highest proportions of fungi at thread-cutting sites and grinding sites fell within the 2.1–3.3 µm size range, at 24.3 and 23.8%, respectively. As shown in Fig. 3, the lowest proportion of fungi at thread-cutting sites was in the 4.7–7.0 µm size range (12.0%). Fig. 4 shows that the lowest proportion of total culturable fungi obtained from air samples in grinding sites fell within the size range ≥7.0 µm (11.4%) occurred. At thread-cutting sites, *Cladosporium* was the dominant fungal isolate in the 2.1–3.3 µm size range, followed by non-sporing and *Mucor* (Fig. 3). At grinding sites, a similar fungal distribution occurred in the size range of 2.1–3.3 µm. As shown in Fig. 4, *Cladosporium* was the dominant fungal isolate in the 2.1–3.3 µm size range, followed by *Mucor* and non-sporing at grinding sites.

The levels of airborne fungi of both 3.3–4.7 and 0.65–1.1 µm at thread-cutting sites were statistically different from those found at grinding sites ($P = 0.002$). In addition, the concentration of *Mucor* in the size ranges of 0.65–1.1, 1.1–2.1, 2.1–3.3,

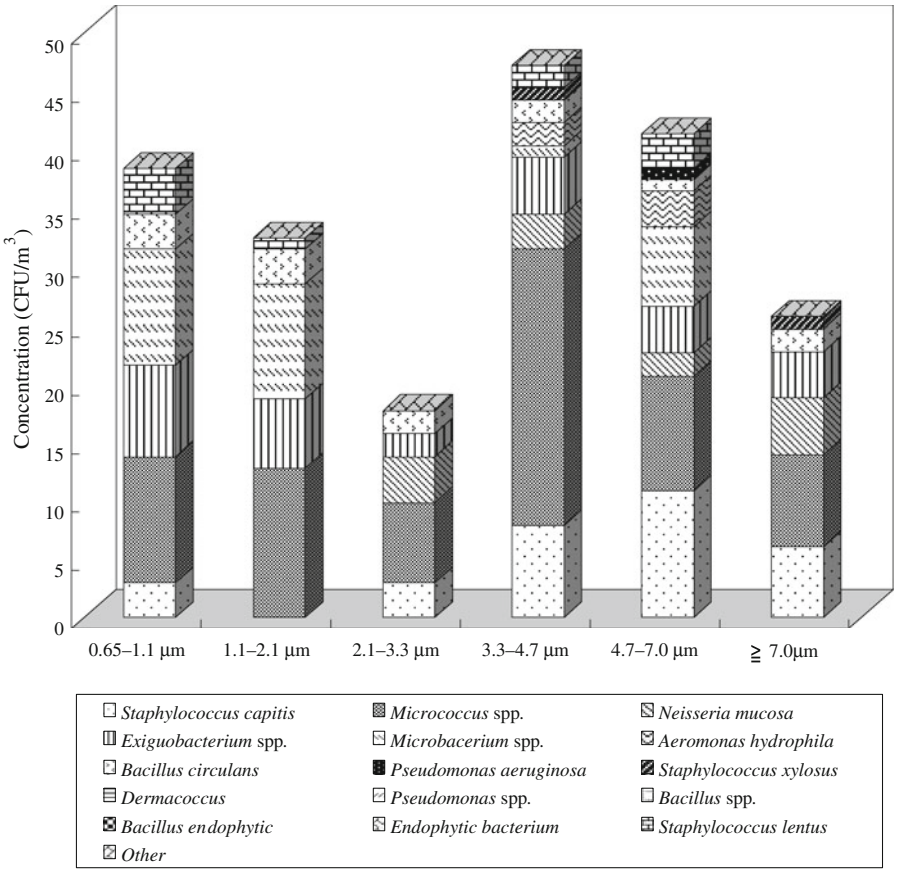

Fig. 2 The species, concentrations, and size distribution of total bacteria at the grinding sites

3.3–4.7, and ≥7.0 μm showed statistical difference between the types of sites ($P < 0.05$). Levels of both *Penicillium* and non-sporing fungi in the size range of 0.65–1.1 μm were statistically different.

The dominant size range for total outdoor bacteria was 1.1–2.1 μm (19.2%), and the lowest proportion of total culturable bacteria fell within the size range 4.7–7.0 μm (14.1%). The highest proportion of fungi in outdoor air samples fell in the 3.3–4.7 μm (21.6%) size range, and the lowest proportion of total culturable fungi fell within 2.1–3.3 μm (13.0%).

It is well known that the size of most of bacteria are smaller than fungi; however, the aerodynamic particle size range of culturable bacteria was greater than culturable fungi, at thread-cutting and grinding sites ($P < 0.01$) in the findings, according to Mann–Whitney tests to compare between size distribution between bacteria and fungi. The results are explained that bacteria in MWF environments can stick to other particles to aggregate into larger particles (Wang et al. 2007).

3.4 Analysis of culturable bacteria and fungi in MWF sumps

In this study, the mean concentration of total airborne fungi of MWF sumps from six thread-cutting machines was 1.7×10^5 ($\pm 0.3 \times 10^5$) CFU/ml. The predominant fungi identified were *Aspergillus* (41.0%), *Penicillium* (19.7%), and yeast (15.4%), with mean concentrations of 7.4, 7.2, and 2.7×10^5 CFU/ml, respectively. Similarly, the mean concentration of fungi of the MWF sumps from six grinding machines was 1.7×10^5 ($\pm 0.1 \times 10^5$) CFU/ml. The dominant fungi identified were *Aspergillus* (41.0%), *Penicillium* (19.7%), and yeast (15.4%), with mean concentrations of 7.2, 5.9, and 3.5×10^5 CFU/ml, respectively. On the other hand,

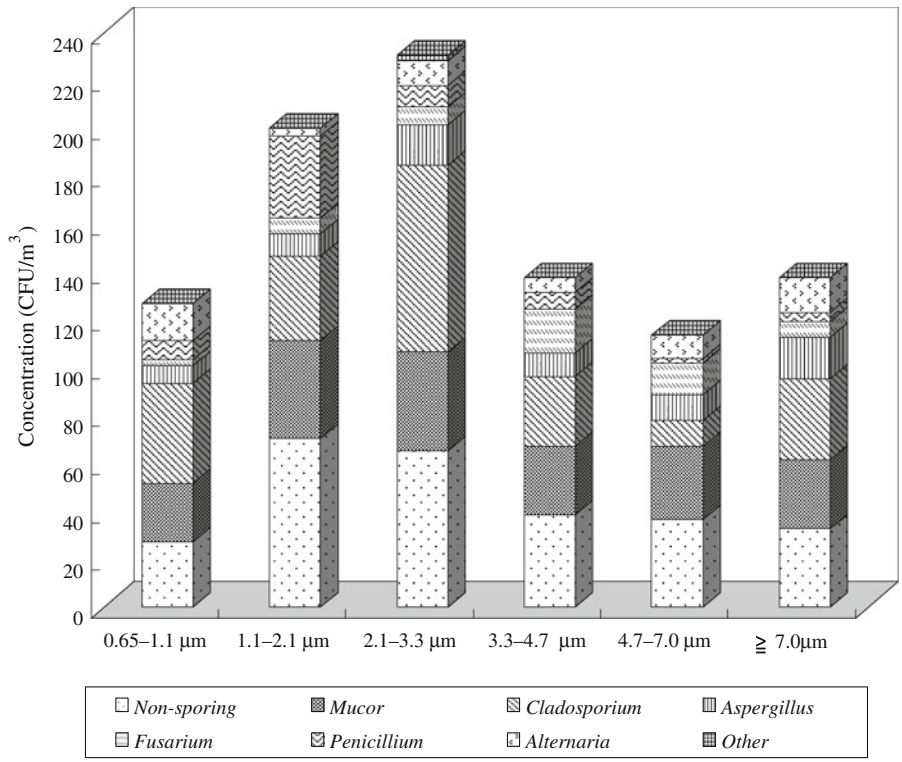

Fig. 3 The species, concentrations, and size distribution of total fungi at the CNC thread-cutting sites

the mean concentrations of bacteria in the MWF sumps from six thread-cutting machines and six grinding machines were similar, at 3.9×10^5 ($\pm 0.6 \times 10^5$) CFU/ml and 3.2×10^5 ($\pm 0.7 \times 10^5$) CFU/ml, respectively. *Pseudomonas* spp. was the only identified bacteria in the MWF sumps from two different machines.

4 Discussion

In this study, the number of genera of Gram-negative bacteria was higher than that of Gram-positive bacteria, whereas the most abundant airborne genera found were Gram-positive bacteria, especially *Exiguobacterium* spp., *Micrococcus,* and *Staphylococcus*. These species most likely came from the work environment (*Bacillus* and *Exiguobacterium*) and the workers' microflora (*Staphylococcus* and *Micrococcus*). The indoor-to-outdoor ratios for the two predominant Gram-positive genera were greater than 1, suggesting that the source of the bacteria was indoors. In addition, this study indicated that between the two different metalworking operations, levels of airborne bacteria at thread-cutting sites were higher than at grinding sites. Many Gram-negative bacteria were identified at the two sites, such as *Pseudomonas* spp., *Aeromonas hydrophila*. Several studies have found that various *Pseudomonas* species are the most common bacterial strains isolated from the metalworking fluid environment, including *Pseudomonas pseudoalcaligenes* (Mattsby-Baltzer et al. 1989a) and *Pseudomonas aeruginosa* (Dilger et al. 2005; Karadzic et al. 2006). However, the present study found *Exiguobacteria* as the most common bacterial species and not *Pseudomonas*. As both *Pseudomonas* spp. and *Exiguobacterium* have been identified as causes of human illness, these findings suggest that a better understanding of MWF microbial ecology is important for workers' health.

Additionally, our results are similar to previous findings that microorganisms such as *Staphylococcus capitis* and *Mycobacterium* spp. exist in water-based MWFs (Bernstein et al. 1995; Veillette et al. 2004; Khan et al. 2005). Several recent reports have potentially linked *Mycobacterium* spp. identified in industrial MWFs with respiratory illness (Chang et al. 2004). For example, *Mycobacterium chelonae* has

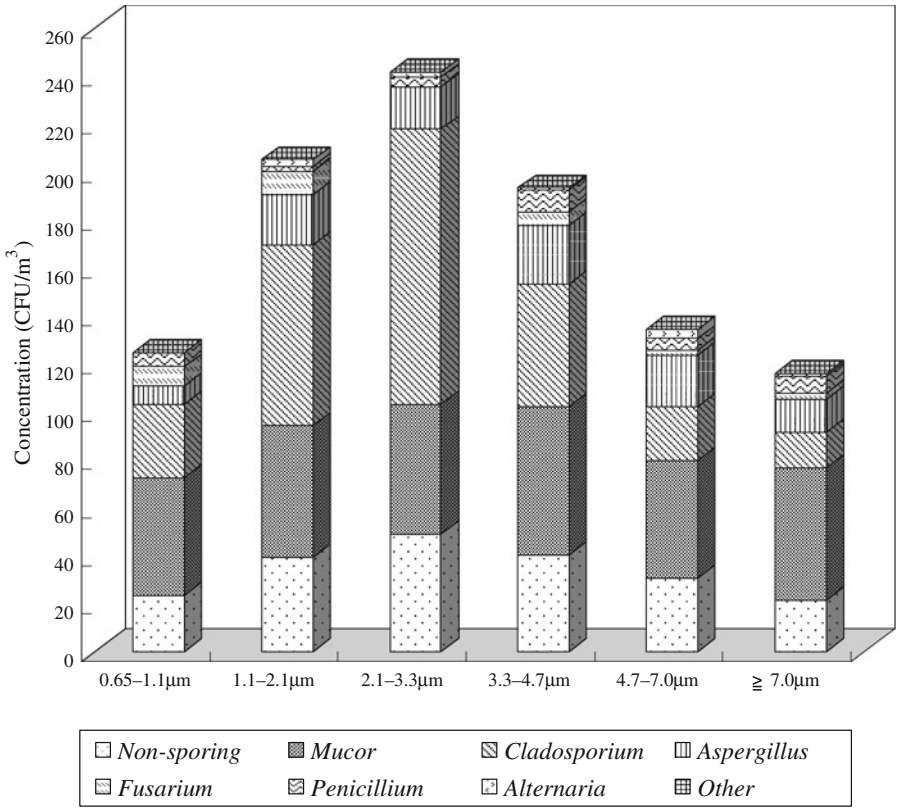

Fig. 4 The species, concentrations, and size distribution of total fungi at the grinding sites

been recovered from MWFs associated with HP (Kreiss and Cox-Ganser 1997; Moore et al. 2000). Due to these issues, *Mycobacterium* spp. has emerged as a source of significant health effects on workers in MWF environments.

Ventilation systems also influence the species and concentrations of bioaerosols, especially the concentrations of bacteria (Tsai and Liu 2009). The thread-cutting sites did have the highest RH, but this was mainly due to the large amount of water-based MWFs and not necessarily (or only) poor ventilation. However, we deemed the staff office inadequately ventilated because it had a high concentration of *Staphylococcus capitis*. The dominance of Gram-positive *Staphylococcus* species has been shown to be an indication of inadequate ventilation, so our data indicate that the staff office was inadequately ventilated (Morey et al. 1986). Inadequate ventilation means a small room with a relatively poor local ventilation system, which was quantified in our study by *Staphylococcus capitis* concentration.

Data regarding the elemental composition of atmospheric aerosols at a given location are widely used to characterize local air quality. Generally, the effects of fungal spores on human health depend on their concentrations, compositions (genera and species), and sizes. Many fungal species are regarded as producers of type I allergens (IgE-binding allergens), and IgE sensitization to common fungal genera, such as *Cladosporium*, *Alternaria*, *Penicillium,* and *Aspergillus,* has been found to be closely associated with allergic respiratory diseases, including asthma (Douwes et al. 2003). Therefore, the higher concentrations of *Cladosporium*, *Alternaria,* and *Aspergillus* that occurred at thread-cutting sites indicate that thread-cutting workers are exposed to a higher risk of human health effects than grinding site workers. Additionally, grinding was performed in a workplace environment with inadequate ventilation, and the common allergic fungi *Cladosporium* and *Aspergillus* dominated the identified fungal species, in agreement with a previous report (Tsai and Liu 2009). This

result has important implications for evaluating the potential risk of exposed workers at the grinding sites. Staff working at the grinding site is at risk of exposure to bioaerosols, so increased ventilation is recommended to minimize the biological hazard at the grinding sites.

Microbial concentrations in MWFs and biosusceptibility may vary depending on the type of operation (Rossmoore 1981; Rossmoore and Rossmoore 1996). In general, exposure to the resulting aerosols is likely to be highest in operations that involve high-speed tools or deep cutting using MWFs, particularly when these processes are not enclosed or ventilation is inadequate. This result is consistent with the higher concentrations of fungi and bacteria observed at thread-cutting sites in this survey. According to the study carried out by Piacitelli et al. (2001), machines without enclosures are more hazardous in terms of exposure to MWFs than fully or partially enclosed machines. In contrast to workers who perform different tasks throughout the day, thread-cutting workers generally perform their tasks and stand near their machine until the pressing cycle is complete. Therefore, these CNC thread-cutting workers spend more time near their machines than workers operating manual machines do (Ross et al. 2004). Because MWFs are generally applied via a jet or as a spray during high-speed machining, even though thread-cutting machines are enclosed, these workers are at increased risk of daily exposure to MWFs.

Aerodynamic diameter is critical in assessing respiratory exposure to bioaerosols, especially for the metalworking environment. This study observed that the size distribution of bacteria at CNC thread-cutting sites fell in the lower range, suggesting that enclosed and vented machines are free of large particles, which agrees with a previous report (Dasch et al. 2005). In addition, the larger bacteria appeared to settle, whereas the smaller bacteria remained suspended in the air. Smaller bacteria, such as *Micrococcus* spp. and *Staphylococcus capitis*, which were the dominant bacterial species in the air at the thread-cutting and grinding sites, could potentially penetrate the alveoli and cause disease. Therefore, the dominant bacteria occurring at these sites are small enough to produce bioaerosols and are known allergens and pathogens that could trigger allergic alveolitis.

According to Park et al. (2001), the type of machine tool (grinder or not) was not associated with MWF sump microbial concentrations. Our results confirmed this conclusion, the total bacterial and fungal counts in MWF sump/fluid were similar. In this study, the total bacterial count and fungal count in the MWF sump/fluid reached levels of 10^5 CFU/ml. However, our results were far lower than those obtained by Mattsby-Baltzer et al. who found levels of 10^8 CFU/ml. In addition, they found *Pseudomonas pseudoalcaligenes* in the area of metalworkers exposed to MWFs associated with respiratory illnesses (Mattsby-Baltzer et al. 1989b). In this study, *Pseudomonas* spp. was the only bacterial isolate obtained from MWF sumps. Therefore, the results of this study suggest this workplace environment poses a potential risk of MWF sumps. In addition, the standard methods for cleaning MWF systems are inadequate, as residual bacteria in the system can rapidly repopulate the newly changed MWF (Veillette et al. 2004). Therefore, workers at these sites are recommended to wear breathing protection.

5 Conclusions

In this study, levels of airborne bioaerosols (bacterial and fungal) at thread-cutting sites were higher than those found at grinding sites. The results of this study may not be representative of what is typical for thread-cutting and grinding procedures. This study revealed that the type of machine tools was not associated with MWF sump microbial concentrations. In addition, the potentially hazardous biological effects resulting from exposure to MWF sumps should be evaluated. Many of the isolates are fairly well known in metalworking environment, but this study reveals certain unusual isolates, like *Exiguobacterium* spp., that are hazardous from the point of view of the health of the machinists. Therefore, we recommend that workers at the CNC thread-cutting sites wear breathing protection and that the local ventilation systems in the staff office be improved to minimize biological hazards. The results obtained from this study may provide useful information for socket-manufacturing industries to implement suitable control measures to reduce workers' exposure to bioaerosols.

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