Characterization of ambient bioaerosols in Singapore

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ABSTRACT

An extensive field investigation was undertaken in Singapore from July through October 2002 on a day-to-day basis to determine the temporal variation of culturable bioaerosols, their particle size distribution and the effect of meteorological parameters on their levels in the ambient air. During the study period, several smoke haze episodes were experienced in Singapore due to the long-range transport of biomass burning emissions from Indonesia. Bioaerosols, sampled during both haze and non-haze periods, predominantly existed in the size range between 1.1 and 2.1 μm. Changes in meteorological conditions had a significant impact on the temporal variation of bioaerosol levels in the ambient air within a day and also from day to day. Microbiological, morphological and biochemical techniques were employed to identify the types of bacteria and fungi present in the ambient air. There were notable differences in the quantity and types of airborne bacteria and fungi between hazy and normal weather conditions. Results indicated that the predominant bacterial genera were *Bacillus* and *Staphylococcus* in the ambient air whereas the predominant fungal genera were *Curvularia* and *Penicillium* during normal weather conditions and *Penicillium* during hazy days.

INDEX TERMS

Bioaerosols; Aerosols; Air pollution; Air quality; Airborne bacteria

INTRODUCTION

In recent years, urban air quality has received considerable attention because of its adverse effects on the human health. Increasing concentrations of airborne particulate matter of chemical and biological origin play an important role in the air hygiene of indoor as well as outdoor environments. Atmospheric fine particles (less than 2.5 μm in diameter) are easily inhaled deeply into the human lungs where they remain embedded for a longer period of time or can be absorbed into the blood stream, and can thus lead to adverse health effects. Over the years, much research has been carried out to determine the sources and fates of chemical contaminants in the airborne particles. However, little research has been done on bioaerosols, especially in tropical environments, although their ability to cause infectious diseases or trigger allergic reactions in humans is fairly established by now (Reponen *et al*., 2001).

Most of the terrestrial surfaces can be considered as major potential sources of bioaerosols, produced at the ocean–atmosphere and soil–atmosphere interfaces. All natural waters as well as anthropogenic waters (sewage lagoons, cooling towers) contain large number of microorganisms. Therefore, water or liquid droplets resulting from rain, splashes or bubbling processes may contain bioaerosols, which may remain airborne after the water evaporates (Nevalainen *et al*., 1993). Apart from these sources, bioaerosols are released from human activities such as agricultural processes, industrial activities as well as solid waste disposal, waste treatment, from activities at homes, work places, schools, hospitals, etc.

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Since Singapore is one of the densely populated countries in the world, the biological quality of air and its influence on human health are of major concern. Biological particles in outdoor air tend to affect the indoor air quality as well because these particulates can enter air-conditioned buildings through the fresh air intake and/or by infiltration through openings and gaps in buildings. Investigation of the biological quality of the ambient air in Singapore, during the hazy days, is particularly very important due to the fact that there is a significant increase in the number of respiratory illnesses and inflammatory diseases during smoke haze episodes in the region. An extensive field investigation was conducted at NUS from July through October 2002 on a day -to-day basis to determine the particle size distribution of ambient bioaerosols (bacteria and fungi), their concentrations on normal and hazy days, and the diurnal variability of size-differentiated bioaerosols under a variety of weather conditions. In addition, the types of bacteria and fungi in ambient air were identified at the genus level. Results obtained from this extensive field study are presented and discussed in this communication.

METHODS

Description of the Sampling Site

Bioaerosol sampling was conducted at the rooftop of the building E2, National University of Singapore (Latitude 1°18′N, longitude 103°46′E). The site is at an altitude of 67 m above the sea level. There were no local sources of pollution in the immediate vicinity of the sampling site. Air sampling was usually carried out during the day time from 8.00 a.m. to 6.00 p.m.

Method of Sampling

The aerosol sampling instrument that was used to collect the air samples in this study was the Anderson six-stage Cascade Impactor with aerodynamic cut size diameters of 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 μm with an airflow rate of 28 l/min. Bioaerosol samples were collected with three different sampling frequencies depending on the weather conditions: every 3 h, every 2 h or on an hourly basis. A total of 188 air samples were collected during this study period. Two impactors were used at the same time, side by side, for the investigation of bacterial and fungal counts present in the ambient air. During the hazy days, intensive air sampling was done on an hourly basis. Altogether, 58 air samples were collected during the haze period. Over the sampling period, meteorological parameters were also recorded in order to investigate the relationship between bioaerosol counts and the meteorological conditions.

Two different collection media were used for the selective sampling of airborne bacteria and fungi. While Trypticase Soy Agar (TSA) was used for the cultivation of bacteria with cycloheximide to inhibit the fungal growth, Malt Extract Agar (MEA) was used for the cultivation of fungi with chloramphenicol to inhibit the bacterial growth. TSA plates with collected samples were then incubated in inverted position for 2 days at 35°C whereas MEA plates were incubated for 2–5 days at 25°C. After the incubation, the number of colonies in each plate were counted and recorded. The counts are presented in colony forming units per cubic meter (CFU/m^3).

Isolation and Identification of Culturable Bioaerosols

Morphologically different individual colonies in selected air samples, collected on TSA during normal days as well as on hazy days, were sub-cultured onto the TSA medium and incubated for 2 days at 35°C until the pure cultures were obtained. Morphological, cultural and biochemical characteristics were used in the identification process.

Morphologically different fungal colonies in selected air samples collected were sub-cultured onto the MEA medium and incubated for 2–5 days at room temperature until the pure cultures were obtained. Identification of isolated fungi was performed using the Agar Block Slide Culture Technique. The staining reagent used in this method was the Lactophenol Cotton Blue. The identification of individual fungi was based on the morphological characteristics and colony characteristics. Spore structures of the fungi were noted and images of the fungal structures were captured in photographs for identification purposes.

RESULTS AND DISCUSSION

Size Distribution of Airborne Biocontaminants

Figure 1 shows the typical size distribution for bioaerosols in Singapore based on repeated field measurements over nine consecutive days; the error bars indicate the standard deviations resulting from repeated measurements. Most of the outdoor airborne bacteria and fungi were in the particle size range 1.1–2.1 µm, followed by 2.1–3.3 µm. About 55% of total culturable airborne bacteria and 72% of total culturable airborne fungi are found in these particle size ranges. This observation is consistent with those reported in the literature for airborne microorganisms in other tropical environments (e.g. Lin and Li, 1996). However, it should be pointed out that these particle size distributions tend to vary significantly within a day depending on the prevailing meteorological conditions.

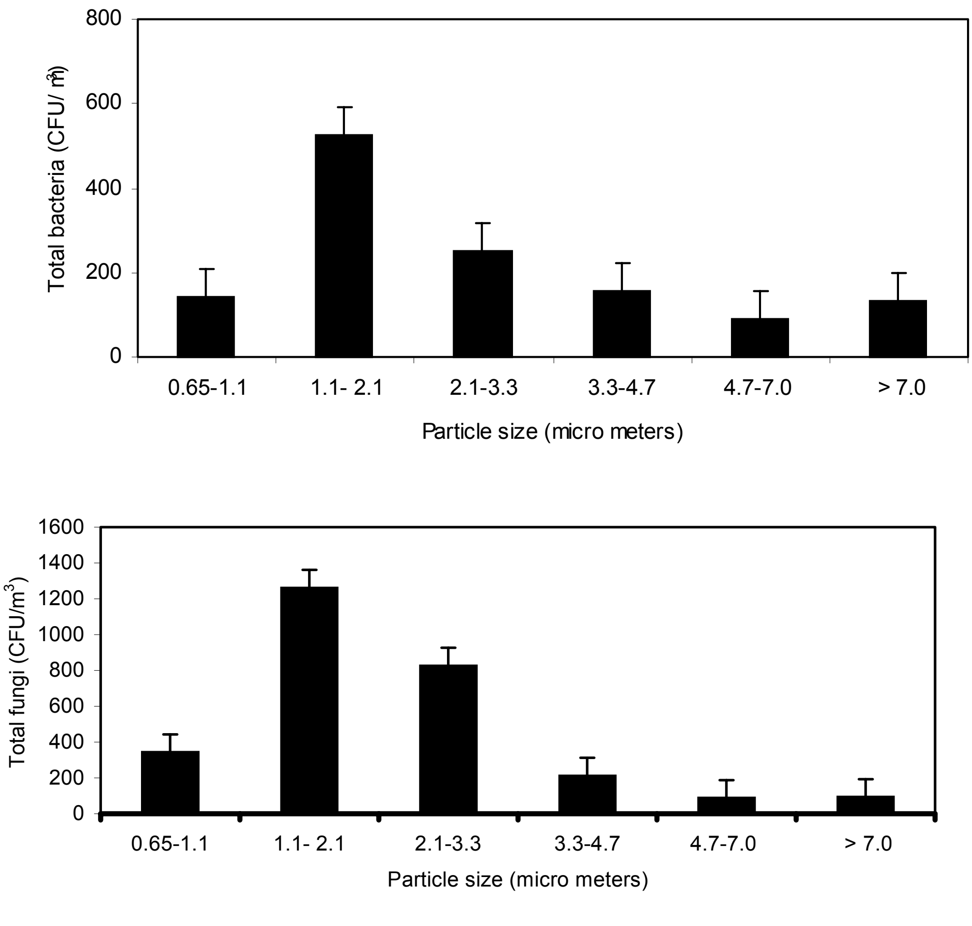

Figure 1 Average size distribution of airborne bacteria and fungi within a day.

Airborne Bacteria and Fungal Concentration Profiles

A typical profile with the temporal variation of airborne bacterial and fungal counts obtained during the daytime is shown in Figure 2. There are three interesting features in this figure. First, the total count of total culturable fungal aerosols is larger than that of bacterial aerosols throughout the measurement period. Second, the maximum bacterial concentration occurs at 10.00 a.m., while fungal concentration has a peak at 9.00 a.m. Third, the concentrations of biological contaminants decrease in the early afternoon and then remain low. The temporal variation observed in this study is somewhat similar to those reported by Li and Li (2000), who demonstrated that high fungal concentrations occurred in early morning and in late afternoons in Taiwan.

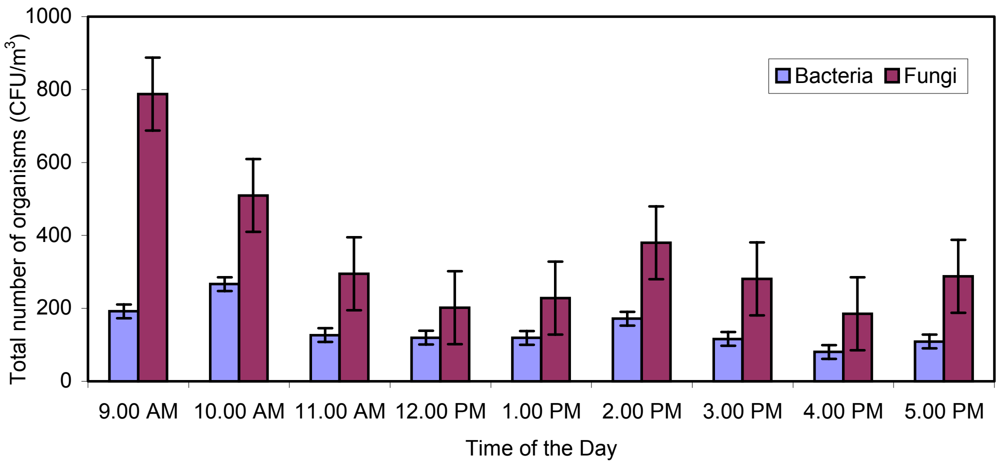

Figure 2 General daily profiles of airborne bacteria and fungi concentrations.

However, in the present study the late afternoon counts were rather low compared to those obtained during the early morning hours. The difference in the temporal variations between these two studies appears to be mainly influenced by changes in the meteorological conditions during the day.

Somewhat elevated concentrations of airborne bacteria and fungi observed during the morning hours are of particular concern because of the following reasons. The ambient air in Singapore is not well dispersed until around the noontime, leading to the accumulation of air pollutants including bioaerosols. As discussed earlier, the maximum concentrations of both fungal and bacterial aerosols are found in inhalable size ranges of particles (<3 µm). These smaller sized particles can penetrate deeply into the lungs of the humans, and no known respiratory mechanism can remove these particles from the respiratory system easily. Therefore, people exposed to relatively high bioaerosol concentrations during the morning hours may develop allergic, toxic and even infectious responses, especially those individuals with immunodeficiency, depending on the type and amount of specific biocontaminants found in the air.

Influence of Meteorological Parameters on Levels of Airborne Bacteria and Fungi

As part of this study, the relationships between bioaerosols and meteorological factors were investigated by continuously monitoring the changes in atmospheric conditions during the course of air sampling. Temperature, relative humidity of air, the solar radiation, rainfall, wind speed and direction are among the meteorological parameters that were continuously tracked at the sampling site.

Results of the present study clearly indicate that there is an inverse relationship between bacterial counts and the solar radiation. When there was a low solar radiation especially in the morning, the bacterial counts were high, whereas at noon the counts were low due to a considerable increase in the solar radiation intensity. A number of laboratory studies with pure cultures have convincingly demonstrated the bactericidal properties of solar irradiation (e.g. Pacia *et al.*, 2001). This suggests that various bacterial species exposed to sunlight become sterilized in a relatively short time. The lethal effect of sunlight is associated with the ultraviolet region of the solar spectrum, whereas wavelengths in the red and infrared regions are generally considered to have little effect other than heating. Although solar radiation damage to microorganisms is known through the laboratory studies with pure cultures, that with the ambient populations present in the atmosphere has only been reported in the literature (Xuejun *et al.*, 1999; Lin and Li, 2000). The observations made in this study are thus consistent with those reported in the literature. Solar radiation affects the fungal concentrations in a similar way as with bacteria. An increase in the solar radiation intensity leads to a decrease in the total culturable fungal counts. The overall effects of solar radiation on airborne bacteria and fungi concentrations depend on many factors including bacterial and fungal species in the ambient populations, the physiological state of the organisms, light intensity/duration (fluence) and wavelengths of the sunlight.

As for the effect of temperature on airborne biological contaminants, both bacterial and fungal concentrations decreased as the ambient temperature increased. This is to be expected as solar radiation and ambient temperature are closely related to each other. During the course of this study, it was also noted that the total bacterial counts were higher on cloudy or rainy days than on sunny days This is due to the fact that on cloudy/rainy days the temperature of the atmosphere is not so high as on sunny days, and at the same time the solar radiation is also lower than on sunny days. An increase in the atmospheric temperature can affect biological properties related to survival (Brown, 1954), and thus lead to the cellular inactivation in the airborne state of bacteria. The effect of the relative humidity on the bacterial and fungal counts was in contrast to those of solar radiation and temperature. When the relative humidity of outside air was high, high bacterial and fungal counts were obtained and vice versa. Relative humidity also plays an important role in the release of spores of many fungi. An increase in the fungal counts was indeed observed at the sampling site after a rain event (28 August 2002). According to the results obtained in this study, most of the times the bacterial and fungal counts were high when the wind blew from the land than from the sea, suggesting that humans and their activities tend to increase the release of bacteria and fungi. An inverse relationship between biological counts and wind speed was observed in that the concentrations of bacteria and fungi were higher under slow moving winds.

Concentration Profile of the Airborne Bacteria and Fungi during Hazy Days

During the course of the present study, moderate hazy conditions, characterized by reduced atmospheric visibility, were experienced in Singapore on several days. The reduced atmospheric visibility was due to the transport of biomass burning (forest fires) emissions from Sumatra, an Indonesian province as revealed by satellite images, trajectory models and weather data. Sumatra is located about a few hundred kilometres away in the southwest direction from Singapore. In general, the air quality in Singapore is affected when the biomass burning emissions are advected towards Singapore and Peninsular Malaysia by the prevailing winds. The combustion of vegetation has now been recognized as a major source of atmospheric pollution because of the release of gases and particles into the atmosphere in considerable quantities. The degradation in the ambient air quality caused by the increased quantities of gaseous and particulate pollutants is of public concern as they have undesirable effects on human health. In the present study, efforts were made to assess the biological quality of the outdoor air during the haze period. On 14, 15 and 25 October 2002, the bacterial and fungal counts were higher than those observed when the weather was normal. In particular, on 14 October the total culturable bacteria and fungi concentrations sporadically increased at about 2 p.m. and remained high during the sampling period. This temporal trend is rather unusual. The PSI (pollutants standard index) recorded on that day was 92, and it was the highest value recorded for 2002. The elevated levels of bioaerosols, together with other air pollutants (chemical pollutants in airborne particles and gaseous pollutants), may have a synergistic effect on human health, especially the sensitive members of the population like the young children, the elderly and people with pre-disposed health conditions.

The predominant bacterial genera isolated under normal weather conditions in the present study were genus *Bacillus* followed by genus *Staphylococcus* and *Micrococcus*. The predominant bacterial genera isolated under hazy conditions in the present study were genus *Bacillus*, followed by the non-saccharolytic non-motile rods. A number of bacterial genera were found in both haze and non-haze conditions. However, the bacterial group, *Enterobacteriaceae*, was only found in haze samples. The fungal genera identified during the normal weather conditions belong to different species of the genera *Curvularia*, *Sporandonema*, *Cephalosporium*, *Helminthosporium*, *Penicillium*, *Trichoderma*, *Cladosporium*, *Aspergillus* and *Botrytis*. The fungal genera identified during the hazy conditions belong to different species of the genera *Curvularia*, *Cephalosporium*, *Penicillium*, *Cladosporium*, *Aspergillus*, *Aurobasidium* and *Sporothrix*.

CONCLUSION

Total culturable bacterial and fungal counts, particle size distribution, and the identification of bacteria and fungi in the ambient air in Singapore were assessed on a day-to-day basis during normal and hazy weather conditions. Meteorological parameters were also monitored during the sampling period in order to assess the effect of meteorological parameters on the bacterial and fungal counts. This study clearly indicates that there is a strong diurnal variation of the total culturable bacterial and fungal counts in Singapore. Particle size distribution of bioaerosols revealed that most of the airborne bacteria and fungi in Singapore are in the inhalable range between 1.1 and 1.2 μm of aerodynamic diameter. By and large, the types of bacteria and fungi during normal weather conditions were different from those identified on hazy days.

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