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AREA 6.5 • HERITAGE CONSERVATION • RESEARCH ARTICLE

Variation of airborne bacteria and fungi at Emperor Qin's Terra-Cotta Museum, Xi'an, China, during the "Oct. 1" Gold Week Period of 2006

Yi-Ping Chen · Ying Cui · Jun-Gang Dong

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Abstract
Background, aim, and scope To stimulate the national economy, a so-called "gold week" comprising May Day and National Day has been put in force by the government, and the first golden-week holiday began on October 1, 1999. Statistical data show that about 15,000 visitors were received each day by Emperor Qin's Terra-Cotta Museum during just such a gold week period. To evaluate the effects of tourism on indoor air, airborne samples were collected by the sedimentation plate method for 5 min during the "Oct. 1" gold week period of 2006, and both composition and changes of airborne bacteria and fungi in indoor/outdoor air in the museums were investigated.
Materials and methods Airborne microbes were simultaneously collected by means of gravitational sedimentation on open Petri dishes. Three parallel samples were collected at the same time each day, and samples were subsequently incubated in the lab. Microbiology media were prepared before each experiment by a professional laboratory. Concentrations were calculated and presented as average data of colony-forming units per cubic meter of air (CFU/m^3).
Results The results show that (1) 13 bacterial genera and eight genera of fungi were identified from indoor and outdoor air at Emperor Qin's Terra-Cotta Museum during "Oct. 1" gold week in 2006. The bacterial groups occupied 61%, the fungi groups occupied 36%, and others occupied 3% of the total number of isolated microorganism genera. (2) As for the comparison of indoor and outdoor samples, the average concentrations of fungi were higher during the afternoon (13:00) than for the morning (09:00). The average concentrations of bacteria in indoor air were higher during the afternoon (13:00) than for the morning (9:00), and in outdoor air, they were lower during the afternoon (13:00) than for the morning (9:00). (3) The average concentrations of five dominant groups of bacteria and three dominant groups of fungi were higher during the afternoon (13:00) than for the morning (9:00) in the indoor air, but the average concentrations of fungi were higher and those of bacteria were lower during the afternoon than for the morning, for outdoor air. (4) As for the comparison of indoor samples, the bacterial daily concentrations and fungal daily concentrations were higher during the afternoon (13:00) than those for the mornings (9:00) over the 10 days. For the comparison of outdoor samples, the bacterial concentration was lower, and the fungal concentrations were higher during the afternoon (13:00) than those for the morning (9:00) over the 10 days.
Discussion The results also show that the numbers of airborne bacteria and fungi had a daily character in indoor air and were higher in the afternoon. The airborne microbe concentrations were found to be similar to residential indoor values from other reports; the indoor museum maximum of microbial concentrations was 90 CFU/m^3 and did not exceed the Chinese indoor bioaerosol guideline. However, microorganisms may fall on the surface of display items as a result of particle sedimentation and

Y.-P. Chen (✉)
SKLLQG, Institute of Earth Environment,
Chinese Academy of Sciences,
No. 10, Feng-Hui Road,
Xi'an 710075, People's Republic of China
e-mail: lifekxyj@hotmail.com

Y. Cui
Xi'an Li-Jun Pharmaceutical Co. Ltd,
Xi'an 710078, People's Republic of China

J.-G. Dong
College of Architecture,
Xian University of Architecture & Technology,
Xi'an 710055, People's Republic of China

would, as such, be capable of degrading objects by way of their secretions, e.g., enzymes and organic acids. Therefore, the right steps should be taken to prevent any deterioration in the quality of displayed artifacts.

Conclusions The results show that museum air was affected by human activity; therefore, it is imperative that the number of visitors be strictly limited and that windows be opened regularly to avoid air pollution.

Recommendations and perspectives The data provide a significant scientific basis for indoor air quality control and museum scientific management. It is recommended that the number of visitors be strictly limited.

Keywords Air microbiology · Air pollution · Bioaerosols · Indoor air · Museum environment · Terra-Cotta museum

1 Background, aim, and scope

On March 29, 1974, local farmers from Lin-tong County discovered by accident some pottery fragments and ancient bronze weapons while drilling a well. Subsequently, an archeological team excavated the Qin Dynasty tomb, which was opened to the public on Oct 1, 1979, as Qin Shi huang's Terra-Cotta Museum. The now well-known Qin Shi huang's Terra-Cotta Warriors and Horses Museum (34° 44′ N, 109°49′ E) is the largest on-site museum in China and is located 30 km east of Xi'an, covering a total area of 16,000 m^2 (see Fig. 1). It contains terra-cotta sculptures depicting more than 8,000 soldiers, 130 chariots, 520 horses, and 150 cavalry horses, made during the Qin Dynasty (221 BC–206 BC). It has been called the Eighth Wonder of the World (Meng and Zhang 2001) and was put on the list of UNESCO world-class cultural heritage sites in 1987. Today, Qin's Terra-Cotta Museum receives about 1.5 million visitors from home and abroad annually.

The quality of indoor air in museums is important for the preservation of antiquities and for tourist health. Research on the indoor–outdoor air pollution in museums has been reviewed by Baer and Banks (1985) and Brimblecombe (1990). Museum atmospheric monitoring has been carried out in many European museums, such as the Correr Museum, Venice (Italy), Kunsthistorisches Museum, Vienna (Austria), Royal Museum of Fine Arts, Antwerp

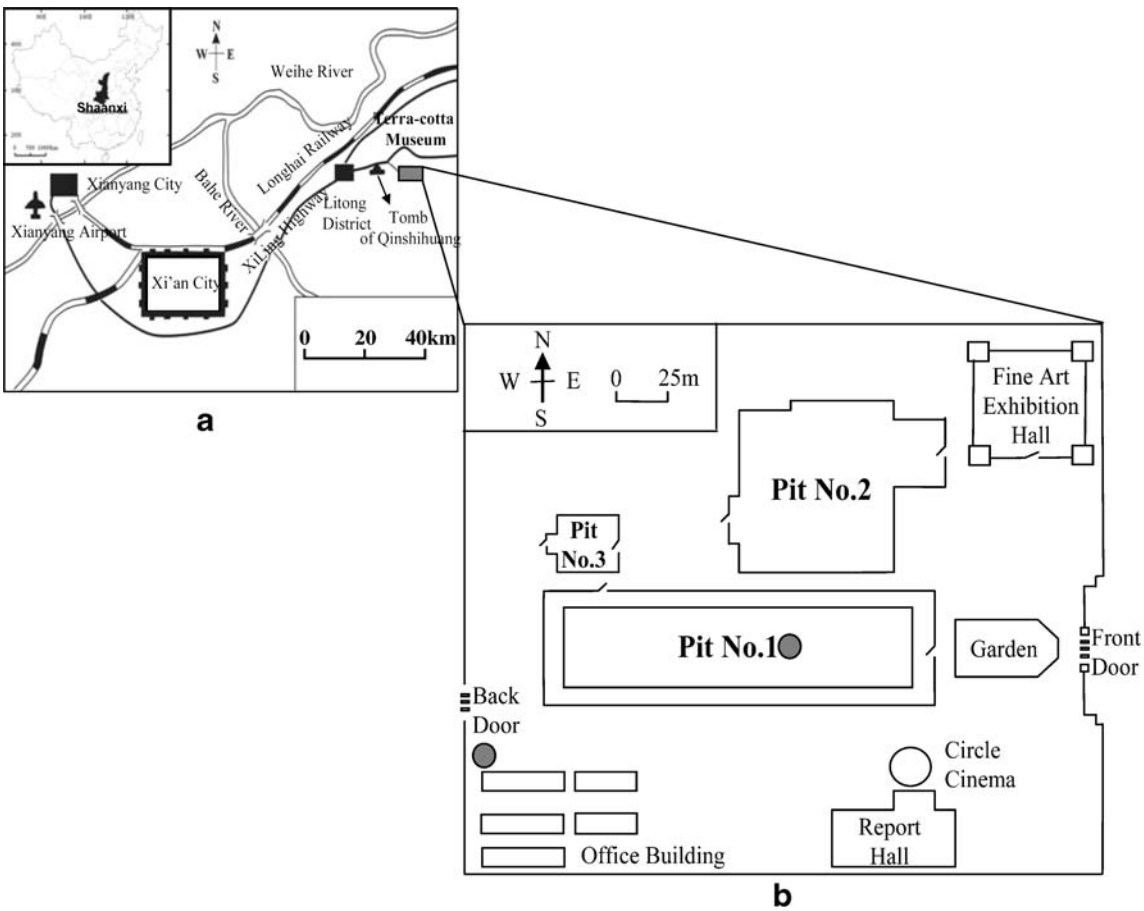

Fig. 1 The locations of the collection sampling sites inside (*gray dot*) and outside (*gray dot*) at the Museum of Qin Terra-Cotta Warriors and Horses in Xi'an, China

(Belgium), and Sainsbury Centre for Visual Arts, Norwich (UK; Brimblecombe et al. 1999; Camuffo et al. 1999, 2001; Gysels et al. 2002). The chemical composition of total suspended particles and PM2.5 (particles with an aerodynamic diameter <2.5 μm) of indoor air in Qin's Terra-Cotta Museum was determined in August 2004 (Cao et al. 2005).

To stimulate the national economy, the "gold week" (a 1-week National holiday), comprising May Day and National Day, has been put into force by the government, and the first golden-week holiday began on October 1, 1999. Domestic scenic spots collected 14.1 billion Yuan (US$ 1.88 billion) from up to 28 million people traveling around China during the first golden-week holiday, while the figures soared to 350 billion Yuan during the 2008 National Day holiday. Statistical data show that about 15,000 tourists visit Emperor Qin's Terra-Cotta Museum each day during gold week periods. However, little is known about the composition and changes of airborne bacteria and fungi in indoor air in museums during gold week periods. It is well known that the earth's atmosphere is teeming with airborne microorganisms. Most airborne bacteria and fungi originate from natural sources such as the soil, lakes, oceans, animals, and humans. Many "unnatural" origins are also known, such as sewage treatment (Adams and Spendlove 1970), animal rendering (Spendlove 1957), fermentation processes (Casida 1968), and agricultural activities which disturb the soil. These organisms are thought to exhibit correlations with air pollution and weather because bacteria and other bioaerosols may attach themselves to other particles and be transported with them (Owen et al. 1992; Chanda 1996). Viable airborne microorganisms are not air pollutants, but should be considered as a factor affecting air quality (Wright et al. 1969). Some airborne bacteria and fungi can be the cause of a variety of infectious diseases as well as allergic and toxic effects. Epidemiological investigations have shown that infectious and non-infectious diseases caused by inhalation of different bioaerosols depend not only on the biological properties and chemical composition but also on concentrations of airborne microbes in the respiratory system (Dales et al. 1991; Husman et al. 1993). Therefore, the aim of this study was to characterize the composition and dynamic change of indoor bioaerosols (bacteria and fungi) in Qin Shi huang's Terra-Cotta Museum during gold week periods in order to provide background information for comprehensive evaluation of indoor air quality and to guide tour management.

2 Experimental methods

Three main buildings of the museum—Pit 1, Pit 2, and Pit 3—were built over the excavated sites between 1974 and 1989. Pit 1 is a huge arch-domed steel structure, located at the center of the museum with an area of 16,000 m^2, containing 1,087 terra-cotta warriors. Two sampling sites were selected for this study, one inside and one outside of Pit 1, as shown in Fig. 1 (gray dot). Airborne microbes were simultaneously collected by means of gravitational sedimentation on open Petri dishes (5-min exposure at about 150 cm from ground level), and this was continued over a 10-day period (from Oct.1 to Oct. 10). Three parallel samples were collected at the same time (9:00 and 13:00) each day, and samples were then incubated in the lab. Microbiology media were prepared before each experiment by a professional laboratory (Bacteria Specific: media composition per 1,000 ml distilled water; beef extract 5 g, peptone 10 g, NaCl 5 g, agar 20 g; Fungi Specific; media composition per 1,000 ml distilled water; glucose 10 g, peptone 5 g, KH_2PO 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, agar 20 g). Pre-prepared Petri dishes were transported and stored under cool conditions, but for not longer than 10 days. The plates with agar media, facilitating bacterial growth and with added cycloheximide to inhibit any fungal growth, were incubated for 48 h at 37°C; the plates with agar media, facilitating fungal growth and with added chloramphenicol to inhibit any bacterial growth, were incubated for 72 h at 25°C. The colony concentrations were calculated and presented as the average mean of units per cubic meter of air (CFU/m^3). Selected bacterial and fungal aerosol samples from indoor and outdoor locations were identified according to the genus level based upon their micro- and macromorphological characteristics, using standard taxonomic keys (Zhang 1990).

Differences between indoor and outdoor samples and between P.M. and A.M. bacterial microbe counts were analyzed using a Mann–Whitney test. The data were presented as mean values ± SD.

3 Results

3.1 Microorganism groups' composition

The viable bacteria of 13 genera and viable fungi of eight genera were identified from inside and outside the Emperor Qin's Terra-Cotta Museum during gold week in Oct, 2006. The bacterial groups with 13 genera occupied 61% of the total number of isolated microorganism genera, the fungal groups occupied 36% of the total number of isolated microorganism genera, and others only occupied 3% of the total number of isolated microorganism genera (Fig. 2). Figure 3 shows the groups, their composition, and the percentage of bacteria and fungi measured. The percentages found for the five dominant groups, including *Staphylococcus*, *Arthrobacter*, *Bacillus*, *Pseudomonus*, and *Micrococcus* were 18%, 17%, 17%, 14%, and 13%, respectively

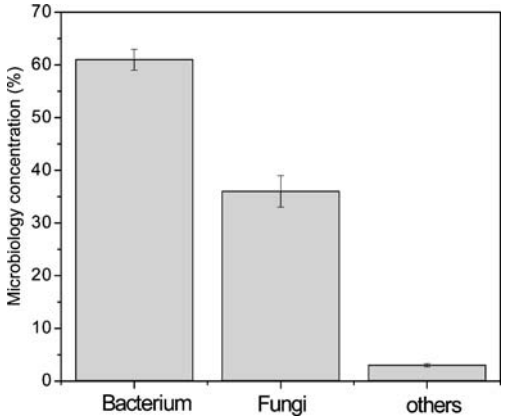

Fig. 2 Microbial composition (as percentage of total concentration) in outdoor and indoor air at Emperor Qin's Terra-Cotta Museum, Xi'an. Data are mean values from 30 independent experiments

(see Fig. 3a). As for the groups of fungi, the percentages of the three dominant groups, including *Penicillium*, *Alternaria*, and *Aspergillus*, were 45%, 31% and 16%, respectively (see Fig. 3b).

3.2 Variation of average concentration of fungi and bacteria

The average concentrations of dominant groups are presented in Fig. 4. As for the comparison of indoor and outdoor samples, the average concentrations of fungi (see Fig. 4c and d) were higher ($P<0.05$) for the afternoon than for the morning. The trend toward a change in the average concentrations of bacteria was not similar to that of fungi. The average concentrations of bacteria in indoor air (see Fig. 4a) were higher ($P<0.05$) during the afternoon than for the morning, and in outdoor air, they were lower ($P>0.05$) during the afternoon than for the morning (see Fig. 4b).

The average concentrations of bacteria and fungi in the outdoor and indoor environment are shown in Fig. 5. The average concentrations of bacteria and fungi were higher ($P<0.05$) during the afternoon (13:00) than for the morning (9:00) in the indoor air, but the average concentrations of fungi were higher ($P<0.05$) and of bacteria were lower ($P>0.05$) during the afternoon than for the morning in outdoor air.

3.3 Daily variation in bacteria and fungi concentrations

Figure 6 shows the trend in daily variation of the bacteria and fungi concentrations in the indoor air and outdoor air during gold week. As for the comparison of indoor samples, the bacterial concentrations (see Fig. 6a) and fungal concentrations (see Fig. 6c) were higher during the afternoon (13:00) than those for the morning (9:00) over the 10-day period of sampling. In the comparison of outdoor samples, the bacterial concentration (see Fig. 6b) was lower during the afternoon (13:00) than those for the morning (9:00) over the 10 days, although the fungal concentrations (Fig. 6d) were higher during the afternoon (13:00) than those for the morning (9:00) throughout the 10-day period.

4 Discussion

Several studies have reported that exposure to large concentrations of airborne microbes is often associated with asthma and rhinitis (Beaumont 1988), hypersensitivity pneumonitis (Siersted and Gravesen 1993), and "sick-building" syndrome (Dales et al. 1991). In addition, exposure to airborne microbes has also been associated

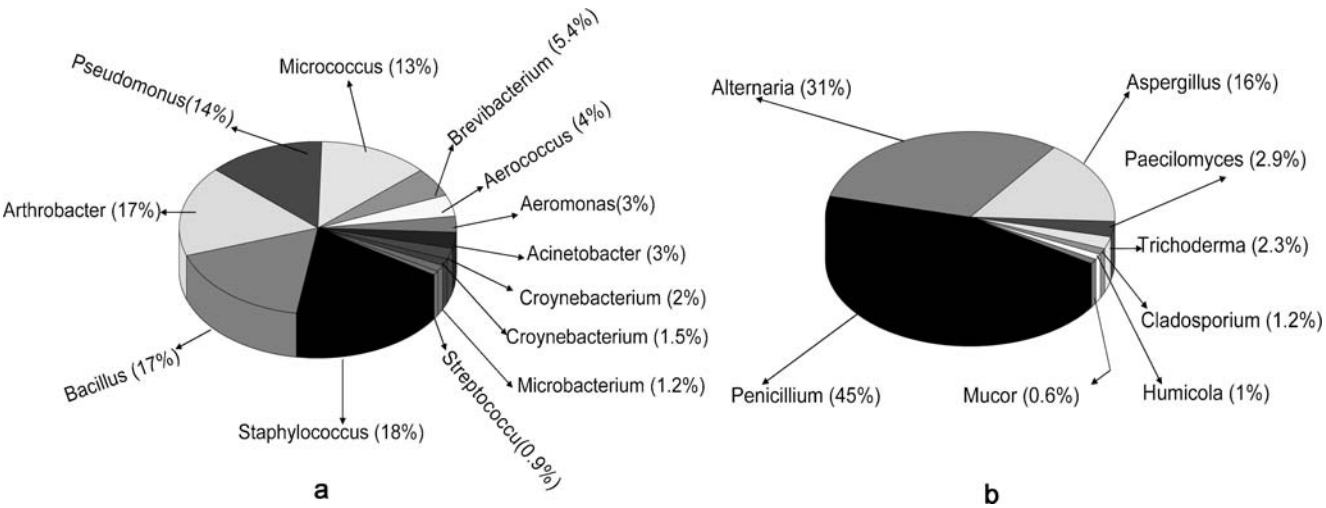

Fig. 3 Community composition of bacteria (**a**) and fungi (**b**) at Emperor Qin's Terra-Cotta Museum in outdoor and indoor air during gold week. Bacteria included 13 genera and fungi included eight genera

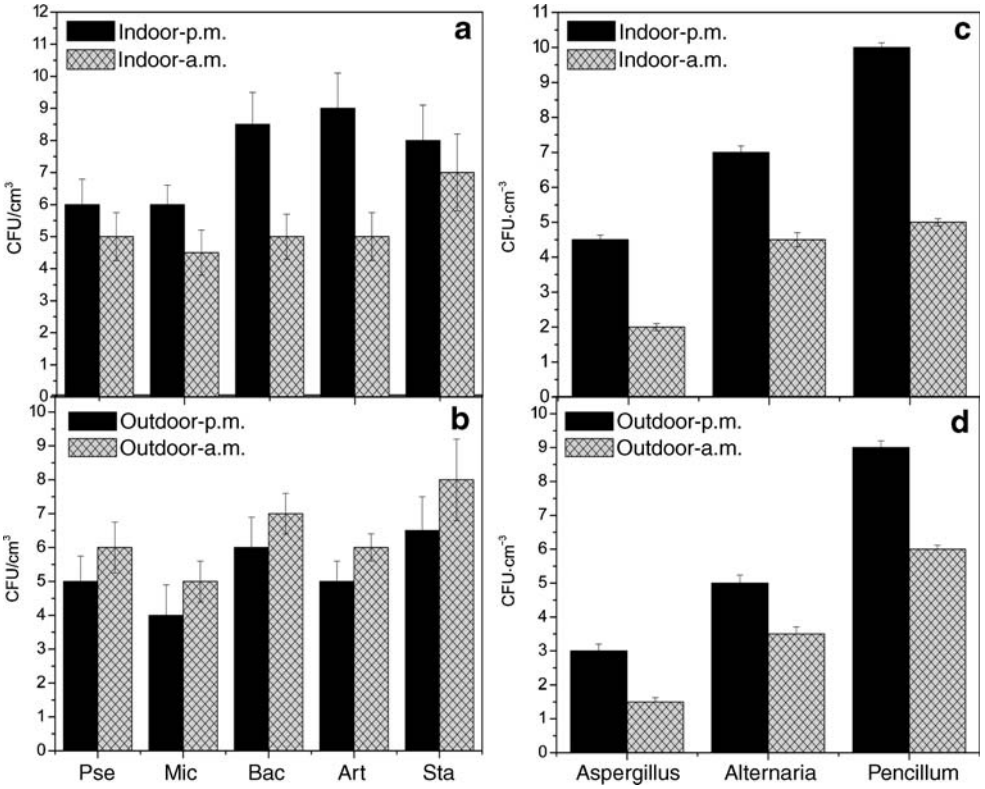

Fig. 4 Variation trends of the average concentration of five dominant groups of bacteria (**a** indoor, **b** outdoor) and three dominant groups of fungi (**c** indoor, **d** outdoor) at Emperor Qin's Terra-Cotta Museum, Xi'an. Data are mean values from 30 independent experiments. In Fig. 3a and b, *Pse* represents *Pseudomonus*, *Mic* represents *Micrococcu*, *Bac* represents *Bacillus*, *Art* represents *Arthrobacter*, *Sta* represents *Staphylococcus*

with a number of other health effects, including infections (Ren et al. 1999). For individual fungi, the threshold concentrations for evoking allergic symptoms have been estimated as 100 *Alternaria* spores per cubic meter air and 3,000 *Cladosporium* spores per cubic meter air (Graveson 1979), while *Aspergillus* spore concentrations above 50 CFU/m^3 have been potentially associated with a higher prevalence of sick-building syndrome (Holmberg 1987). Owing to the ubiquitous presence of airborne microbes in nature, they are essentially present inevitably in most enclosed environments (Pastuszka et al. 2000; Jones and Harrison 2004).

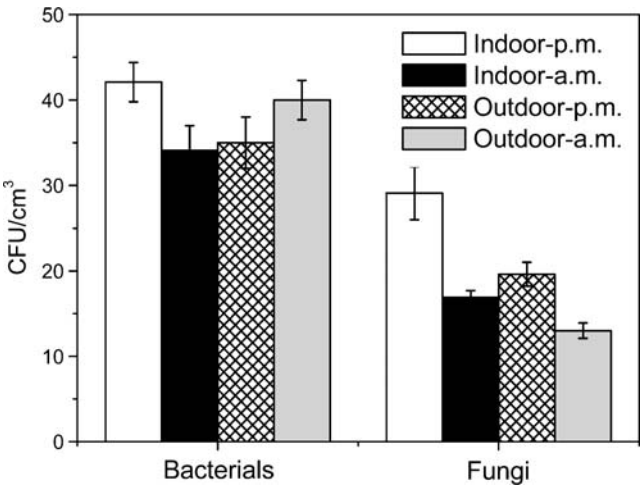

Fig. 5 Average concentration variation for bacteria and fungi in outdoor and indoor air at Emperor Qin's Terra-Cotta Museum, Xi'an. Data are mean values from 30 independent experiments

The Qin Shi huang's Terra-Cotta Museum is a relatively enclosed environment, and about 15,000 tourists visit it every day during the gold week period. Therefore, it is very important to investigate the composition and change in indoor and outdoor air of the Qin Shi huang's Terra-Cotta Museum in terms of the health of the visitors. The viable bacteria of 13 genera and viable fungi of eight genera were identified from inside and outside the Emperor Qin's Terra-Cotta Museum during gold week in Oct 1, 2006. The five most prevalent bacterial genera are consistent with previous studies (Mancinelli and Shulls 1978; Shintani et al. 2004; Shaffer and Lighthart 1997), and the three dominant groups are also consistent with previous studies (Gravesen et al. 1986; Ren et al. 2001; Hong et al. 2003); however, this was not the case for *Cladosporium*.

The results have also shown that the numbers of airborne bacteria and fungi had a daily character in indoor air, being higher in the afternoon. This is most likely related to the number of visitors. The airborne microbe concentrations were found to be similar to residential indoor values from other reports, e.g., bacterial values between 10 and 103 CFU/m^3 (Pastuszka et al. 2000) and total fungal aerosol concentrations in homes ranging from 10 to 103 CFU/m^3 (Kuo and Li 1994; Pastuszka et al. 2000). The indoor museum maximum of microbial concentrations

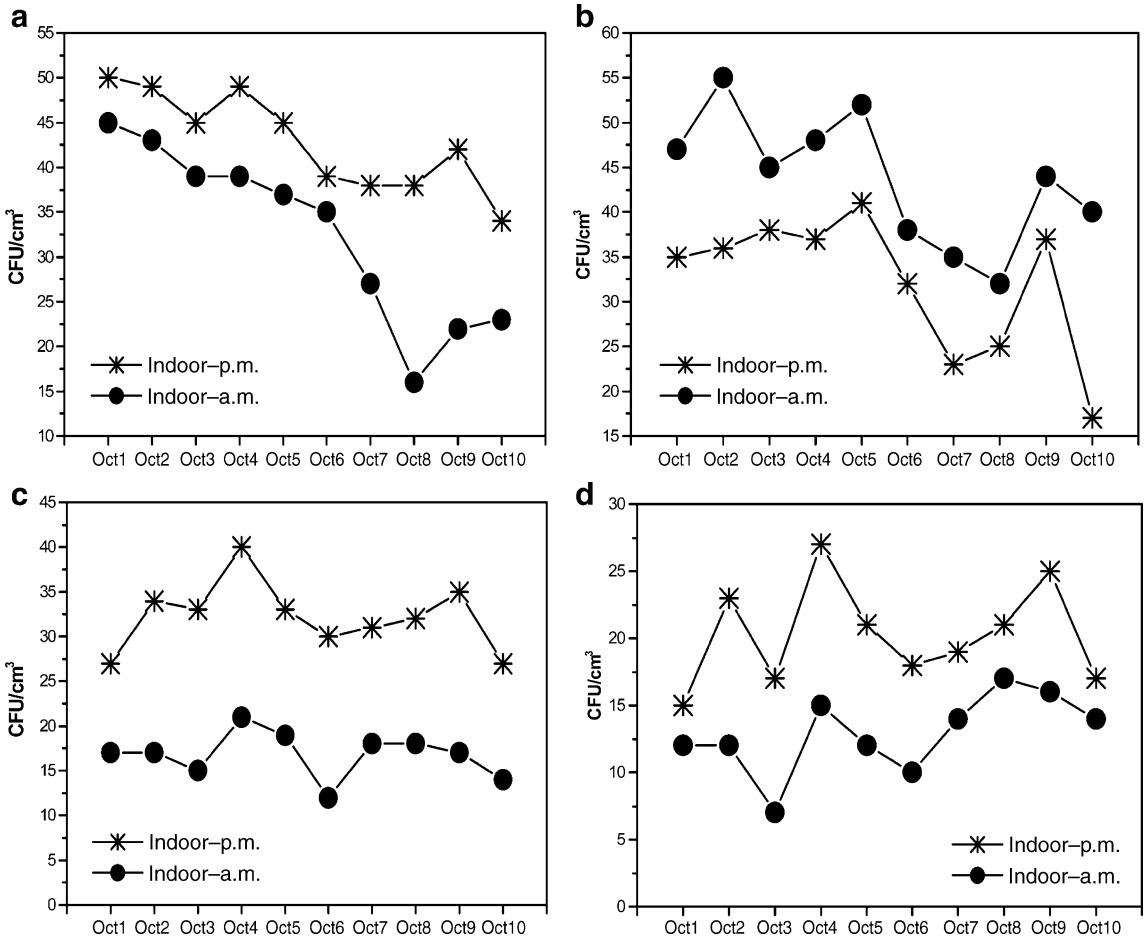

Fig. 6 Daily variation trends for bacterial concentration (**a** indoor—p.m. and indoor–a.m.; **b** outdoor—p.m. and outdoor—a.m.) as well as fungal concentration (**c** indoor—p.m. and indoor—a.m.; **d** outdoor—p.m. and outdoor—a.m.) at Emperor Qin's Terra-Cotta Museum. Data are mean values from three independent experiments

was 90 CFU/m^3, and this does not exceed the Chinese indoor bioaerosol guideline. This value of microbial concentrations might be minimized through our use of sedimentation collection samples. Thus, the results from this study provide comparative data that can be used to better interpret the turbulence arising from visitors to a museum, as well as to improve our understanding of the role of such visitor turbulence in terms of museum air quality. The average concentrations and everyday concentrations of bacteria and fungi were significantly higher during the afternoon than those for the morning in the indoor air. This observation is consistent with the aforementioned reports (Mancinelli and Shulls 1978; Shintani et al. 2004; Shaffer and Lighthart 1997). A possible cause for this result is visitors' turbulence in the indoor air, leading to the resuspension of microorganisms present on the floor, as well as outside air penetration into the museum occurring as visitors travel through the exhibit.

To test our assumed cause as a possibility, outdoor comparison samples were collected when indoor air quality investigations were conducted. The fungi concentrations were significantly higher during the afternoon than those for the morning; conversely, the afternoon concentrations were lower with respect to overall microbe and bacterial counts than those for the morning. The possible explanation for our results was that the ultraviolet radiation in sunlight killed a certain amount of the airborne bacteria, but had no significant effect on airborne fungi. Besides, these bacteria and fungi can potentially damage the museum exhibits (e.g., pottery soldiers, horses, chariots, and weapons) because the microorganisms may fall on their surfaces as a result of particle sedimentation, subsequently degrading objects through the action of their secretions, e.g., enzymes and organic acids. Under normal conditions, bacteria would be unable to grow on the dry surface of paintings, although one could postulate that under extreme environmental conditions they could grow and cause biodecay on these works of arts (Erhardt and Mecklenberg 1994). Therefore, limiting relative humidity becomes an important issue in the preservation of cultural heritage, although both metals and organic materials should be stored within appropriate relative humidity ranges (typically 40–70% organics and 45% metals; Erhardt and

Mecklenberg 1994). According to our records, relative humidity was 55.2% during the "Oct. 1" Gold Week period of 2006. This relative humidity level may possibly have adverse effects in terms of the conservation of the exhibits (e.g., pottery soldiers, horses, chariots, and weapons).

The museum environment is of critical importance for the preventive conservation of the collection and for the visitors' health. Preventive conservation aims to provide an environment appropriate for the storage and display of objects, such that deterioration is either stopped or at least slowed to an acceptable rate. To correct and continue conservation of these unique terra-cotta figures, which are not only of National but also of Global importance in terms of cultural heritage, the right steps should be taken to prevent any deterioration in their quality and composition, while at the same time enabling them to remain on open public display. It is important, firstly, that relative humidity should be reduced; secondly, the ventilation system should be directed onto the terra-cotta figures and switched off during running days; and, thirdly, it is imperative that the number of visitors be strictly limited to avoid air pollution.

5 Conclusions

Exposure to bioaerosols has already been associated with a wide range of health effects, including infectious diseases, acute toxic effects, allergies, and cancer. In particular, exposure to the four most prevalent fungi detected in the present study (*Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria*) has been strongly associated with allergic respiratory diseases (such as asthma). Our investigation has shown that *Staphylococcus*, *Arthrobacter*, *Bacillus*, *Pseudomonus*, and *Micrococcus* were the dominant bacterial groups and that *Penicillium*, *Alternaria*, and *Aspergillus* were the three dominant fungal groups in both indoor and outdoor air. In addition, the results also show that the average concentrations and everyday concentrations of overall microbes, bacteria, and fungi were significantly higher during the afternoon than those for the morning in the indoor air. Accordingly, (1) a future study is recommended to examine the relation between worker exposure to airborne microbes and health effects; (2) it is now necessary to assess the effects of fungal colonization of the terra-cotta sculptures with regard to any influence of fungal metabolism products on pottery soldiers, horses, chariots, and weapons because fungi may fall on their surfaces as a result of particle sedimentation; and (3) it is imperative that the number of visitors be strictly limited and that windows be opened regularly to minimize air pollution.

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