Pergamon

Atmospheric Environment Vol. 30, No. 1, pp. 155–160, 1996
Elsevier Science Ltd
Printed in Great Britain
1352-2310/96 $9.50 + 0.00

1352-2310(95)00143-3

ATMOSPHERIC POLLUTION BY AIRBORNE MICROORGANISMS IN THE CITY OF MARSEILLES

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(*First received* 1 *August* 1994 *and in final form* 21 *February* 1995)

Abstract—Outdoor airborne microflora was investigated in urban and natural areas, the city of Marseilles and the natural reserve of Porquerolles Island, respectively. In Marseilles, concentrations of airborne viable microorganisms averaged 791 ± 598 bacteria m^{-3} (with a geometric mean of 536 ± 103 bacteria m^{-3}) and 92 ± 92 fungi m^{-3} (with a geometric mean of 63 ± 15 fungi m^{-3}). In Porquerolles Island, concentrations of airborne microorganisms reached 42 ± 70 bacteria m^{-3} (with a geometric mean of 26 ± 7 bacteria m^{-3}) and $46 + 55$ fungi m^{-3} (with a geometric mean of 33 ± 8 fungi m^{-3}). Airborne microflora, which followed a log-normal distribution in Marseilles, was shown to have a large variability. Airborne bacteria increased with temperature and wind velocity whereas airborne fungi increased with temperature and varied with wind direction in urban and natural areas. Partial identification of bacteria in Marseilles and Porquerolles Island showed that geographical location had qualitative as well as quantitative influence on airborne microflora, this was illustrated by an increase of global airborne microorganisms, and more particularly Gram negative bacteria, in the urban area.

Key word index: Airborne bacteria, airborne fungi, outdoor air, city, countryside.

INTRODUCTION

Earth's atmosphere is known to teem with airborne microorganisms. Most of them originate from natural sources such as soil, lakes, animals and human. Moreover, industrial operations such as sewage treatment, animal rendering, fermentation processes and agricultural activities also emit viable microorganisms into the air (Bovallius *et al.*, 1978; Spirin and Mikhailova, 1991). Although airborne microorganisms can be found in almost all environments, they do not present a health hazard to the exposed individuals. Nevertheless, most airborne microorganisms have the property of self-replication and can contribute to discomfort humans by causing allergenic symptoms and diseases (Cox, 1989; Flannigan *et al.*, 1991; Xia, 1992). Indoor microflora is reported to be responsible for respiratory and other health symptoms especially among children (Brunel, 1991) and is considered as a direct factor of infection in hospitals (Whyte *et al.*, 1992). The effect of outdoor airborne particles on human health is less evident because of many factors of variation. The number of airborne viable microorganisms has been shown to vary extensively with the type of environment and micro-environment: microbial concentrations are reported to be low over the open sea, somewhat higher over the land and much higher in urban and agricultural areas (Bovallius *et al.*, 1978; Blomquist, 1994). Moreover, atmospheric pollution (carbon monoxide, hydrocarbons, nitric oxide, nitrogen dioxide and sulfur dioxide) (Mancellini and Shulls, 1978) and weather have been shown to influence the survival of microorganisms in the airborne state: temperature, relative humidity and wind velocity have been reported to increase airborne microflora counts depending on sampling sites and microbial species (Jones and Cookson, 1983; Bovallius *et al.*, 1978; Rosas *et al.*, 1993). Thus, the monitoring of current levels of outdoor airborne microorganisms in as many geographical sites as possible appears necessary to evaluate the risk on human health and to study its evolution. The purpose of this study was to investigate the viable population of airborne microorganisms in the city of Marseilles and to compare it to background levels observed in the natural reserve of Porquerolles Island. Moreover, the effect of meteorological factors on airborne microflora was evaluated in both the urban and the natural environments.

MATERIALS AND METHODS

Outdoor concentrations of viable airborne microorganisms were measured approximately 2 days a week in the city

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of Marseilles and in Porquerolles Island, over a 12-month period. For each sampling, airborne microorganisms were collected at 9.00, 12.00, 14.00 and 17.00 h both in Marseilles and Porquerolles Island. Weather parameters such as temperature, relative humidity, wind direction and velocity, were measured simultaneously with each microbial sample, in each sampling site. Due to environmental and technical conditions in Porquerolles Island, no sampling could be performed during rains.

Air sampling procedures

Airborne bacteria and fungi were collected simultaneously by two Reuter Centrifugal Air samplers (RCS +) recently developed from Biotest, which have been chosen for their portability. In Marseilles, the sampling site was located at the University campus, at the city center. Sampling environment was highly heterogeneous: motorways and asphalt-covered parkings on one side, hospital and its vegetated park on the other side. In order to avoid the problem of occasional sources of variation in bacteria and fungi counts such as turbulent air flows due to vehicle traffic or water aerosols due to sprinkler irrigation (Ahmed *et al.*, 1984), the sampling site was located on the roof of the Pharmacy Department, at a height of 50 m above ground level. For each sampling, 160 ℓ of air were aspirated during 4 min, always facing wind directions. In the natural reserve of Porquerolles Island, the sampling site was located at the top of a rock headland (30–35 m above sea level). In order to equal the sampling height of Marseilles, microorganisms were collected at a height of 15 m above ground level. The sampling environment was composed of rocky and sandy areas with a scattered mediterranean vegetation, it was far from any industrial or agricultural activity, in an uninhabitated region. Due to the high variability of airborne contamination in the natural areas, 3 volumes were collected: 120, 360 and 600 ℓ during 3, 9 and 15 min, respectively.

Microbial assay procedures

Airborne particles were collected by impaction onto an agar medium. Aerobic bacteria were enumerated by collecting samples on Trypticase Soy Agar incubated at 30 °C and counting colonies after 2 days. Cycloheximide, final concentration of 0.5 mg ml^{-1}, which has been previously shown not to affect bacterial counts (Bovallius *et al.*, 1978) was added to the media to inhibit growth of fungi. On each plate, approximately 50% of the entire colonies were isolated aimlessly for partial identification by light microscopy based on Gram reaction and bacterial morphology. Fungi were collected on Malt Extract Agar containing chloramphenicol (0.05 mg ml^{-1} final concentration). Fungal colonies were counted after a 5 day incubation period at 25 °C.

Statistical analysis

Distribution analysis was performed by the Chi-squared test. Statistical comparison between airborne contamination observed in Marseilles and Porquerolles Island was performed by the Mann–Whitney rank sum test (one-sided U test). Variation parameters analysis (meteorological factors and time-of-day effects) was performed by the Kruskal–Wallis test. Correlations between bacterial and fungal populations were studied by the rank correlation test of Spearman.

RESULTS

Summary data concerning bacterial and fungal airborne viable particles are reported in Table1. In Marseilles concentrations of airborne bacteria averaged 791 ± 598 CFU m^{-3}, with a geometric mean of 536 ± 103 CFU m^{-3}, and concentrations of airborne fungi averaged 92 ± 92 CFU m^{-3}, with a geometric mean of 63 ± 15 CFU m^{-3}. In Porquerolles Island, concentrations of airborne bacteria averaged $42 + 70$ CFU m^{-3}, with a geometric mean of 26 ± 7 CFU m^{-3}, and concentrations of airborne fungi averaged 46 ± 55 CFU m^{-3}, with a geometric mean of 33 ± 8 CFU m^{-3}. Comparison between viable particles observed in Marseilles and Porquerolles Island by the Mann–Whitney rank sum test showed that airborne microflora was statistically higher in urban area than in natural area, concerning bacteria as well as fungi ($P < 0.001$).

Statistical analysis of distributions by the Chi-squared test showed that bacteria and fungi varied according to a log-normal distribution in Marseilles. Seasonal fluctuations in bacteria and fungal spores counts are reported in Figs 1 and 2: bacteria and fungi levels increased in May and June both in Marseilles and Porquerolles Island.

Concentrations of airborne microorganisms according to time of sampling in Marseilles and Porquerolles Island are reported in Table 2. Statistical analysis by the Kruskal–Wallis test showed that time of sampling had no effect on bacteria ($P = 0.443$ in Marseilles and $P = 0.884$ in Porquerolles Island) and fungi concentrations ($P = 0.725$ in Marseilles and $P = 0.779$ in Porquerolles Island), independently of sampling location.

Statistical analysis of weather effect on airborne contamination is reported in Table 3 concerning the city of Marseilles and in Table 4 concerning the natural reserve of Porquerolles Island. The effect of meteorological factors was shown to be independent of

Table 1. Summary of airborne contamination observed in Marseilles and Porquerolles Island

	Airborne microorganisms in the city of Marseilles (CFU m^{-3})		Airborne microorganisms in Porquerolles Island (CFU m^{-3})	
	Bacteria	Fungi	Bacteria	Fungi
Minimal level	80	6	0	0
Maximal level	2813	556	508	266
Average	791 ± 598	92 ± 92	42 ± 70	46 ± 55
Geometric mean	536 ± 103	63 ± 15	26 ± 7	33 ± 8
Variation coefficient	75%	99%	170%	121%

sampling location. Bacterial concentrations increased with wind velocity and temperature ($P < 0.001$ and $P = 0.04$) in Marseilles as well as in Porquerolles Island ($P < 0.001$ and $P = 0.006$) whereas fungal population depended on wind direction ($P = 0.033$ in Marseilles and $P = 0.036$ in Porquerolles Island) and increased significantly with temperature ($P = 0.015$ in Marseilles and $P < 0.001$ in Porquerolles).

No correlation was found between bacteria and fungi concentrations by the correlation coefficient of Spearman in urban as well as natural areas. Partial identification of bacteria in Marseilles and Porquerolles Island are presented in Table 5. In Marseilles, most airborne bacteria were Gram negative rods (60%) while 80% were Gram positive bacteria in Porquerolles Island.

DISCUSSION

Concentrations of airborne microorganisms observed in Marseilles reached 791 ± 598 bacteria m^{-3}

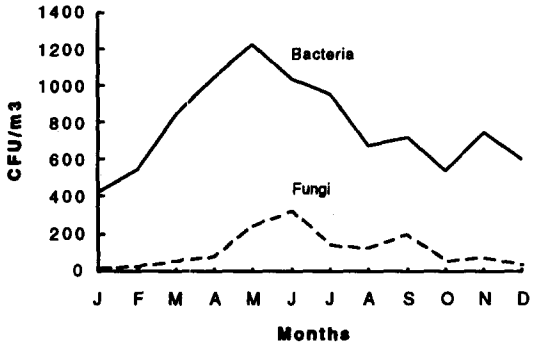

Fig. 1. Seasonal fluctuation of airborne microflora in Marseilles.

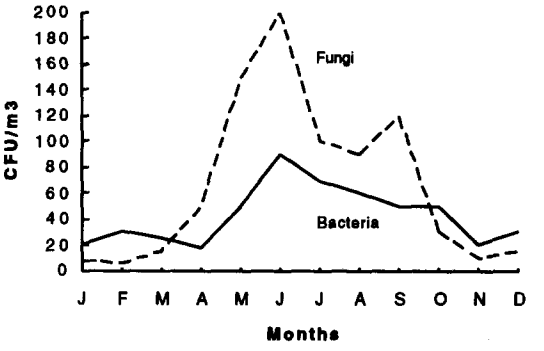

Fig. 2. Seasonal fluctuation of airborne microflora in Porquerolles Island.

Table 2. Effect of time-of-day on airborne microorganisms in Marseilles and Porquerolles Island

Airborne microflora ($CFU\,m^{-3}$)		Time of sampling (h) 09.00	12.00	14.00	17.00	P	Interpretation
Marseilles	Bacteria	901	694	829	703	0.443	Non-significant effect
	Fungi	98	100	73	96	0.725	Non-significant effect
Porquerolles Island	Bacteria	39	58	39	27	0.884	Non-significant effect
	Fungi	45	44	46	47	0.779	Non-significant effect

Table 3. Effect of meteorological factors on airborne microflora in Marseilles

Meteorological factors		Airborne bacteria $CFU\,m^{-3}$	P	Airborne fungi $CFU\,m^{-3}$	P
Wind origin	N–NW	872		105	
	S–SW	670	0.3719	63	0.033*
	E–SE	524		62	
	< 15	673		97	
Wind velocity (knots)	15–25	924	< 0.0001*	62	0.876
	> 25	2069		89	
	< 10	469		25	
	10–15	868		55	
Temperature (°C)	15–20	801	0.040*	91	0.0149*
	20–25	736		134	
	> 25	752		125	
Humidity (%)	< 50	868		96	
	> 50	609	0.5295	84	0.667

* Significant effect ($P < 0.05$).

Table 4. Effect of meteorological factors on airborne microflora in Porquerolles Island

Meteorological factors		Airborne bacteria CFU m^{-3}	Airborne bacteria *P*	Airborne fungi CFU m^{-3}	Airborne fungi *P*
Wind origin	N–NW	65		74	
	S–SW	66	0.931	40	0.036*
	E–SE	60		44	
Wind velocity (knots)	< 15	18		42	
	15–25	38	< 0.001*	46	0.107
	> 25	135		69	
Temperature (°C)	< 10	36		11	
	10–15	68	0.006*	37	< 0.001*
	15–20	88		110	
Humidity (%)	< 50	57		58	
	> 50	53	0.291	47	0.151

* Significant effect ($P < 0.05$).

Table 5. Partial identification of bacteria in Marseilles and Porquerolles Island

Airborne microflora	Marseilles	Porquerolles Island
Gram negative rods	60%	20%
Gram positive cocci	25%	35%
Gram positive rods	15%	45%

and 92 ± 92 fungi m^{-3} and were consistent with previous data where airborne bacteria varied between 500 and 1500 CFU m^{-3} in city parks or city streets (Bovallius *et al.*, 1978; Chihara and Someya, 1989; Jones and Cookson, 1983; Macher *et al.*, 1991; Mancinelli and Shulls, 1978) and airborne fungi averaged 100–1000 CFU m^{-3} in urban areas (Jones and Cookson, 1983; Macher *et al.*, 1991; Pasanen *et al.*, 1990; Rosas *et al.*, 1993). Abundance of airborne contamination in cities can be related to many factors such as vehicle traffic in the streets inducing turbulent airflow, amount of suspended dust and density of people carrying germs. Moreover, bacteria counts have been shown to be strongly correlated with atmospheric pollutants (NO, CO, NO hydrocarbons) which may have a protective effect on microorganisms, depending on microbial species (Lee *et al.*, 1987; Mancellini and Shulls, 1978).

In the natural reserve of Porquerolles Island, airborne bacteria and fungi reached 42 ± 70 CFU m^{-3} and 46 ± 55 CFU m^{-3}, respectively, suggesting that airborne microflora was statistically higher in the urban area than in the natural environment. Similar results were exposed in previous reports where airborne contamination in non-urban environments varied from 10 to 100 CFU m^{-3} concerning bacteria as well as fungi (Bovallius *et al.*, 1978; Chihara and Someya, 1989; Jones and Cookson, 1983), although some authors have observed that the number of airborne fungal spores increased in rural areas (Rosas *et al.*, 1993). Outdoor airborne spores originate from vegetation and soil and their level has been shown to grow in arboreal micro-environments (Nussbaum, 1991). High concentrations of fungal spores in Marseilles could be related to vegetated micro-environments near sampling site. In Porquerolles Island, the absence of arboreal areas near the sampling site could explain low levels of fungal spores in the airborne state.

Frequency distributions of airborne microorganisms in Marseilles could be assimilated to log-normal distributions as reported elsewhere (Bovallius *et al.*, 1978; Chihara and Someya, 1989; Jones and Cookson, 1983). Nevertheless, airborne bacteria and fungi were submitted to high variability in both the urban and the natural areas. This variability agreed with previous reports where standard deviation often exceeded the mean and suggested that biological interactions occurring in the atmosphere are much more complex than it has been previously supposed (Bovallius *et al.*, 1978; Chihara and Someya, 1989; Jones and Cookson, 1983).

The evolution of airborne microflora over a 12-month period showed a seasonal fluctuation in the city of Marseilles as well as in the natural reserve of Porquerolles Island: bacteria and fungi counts increased in summer, with peaks on May and June. Seasonal variation of airborne microflora in various environments has been largely illustrated in previous reports and appeared to depend on sampling sites: most of the authors observed seasonal peaks in summer (Gupta *et al.*, 1993; D'Amato *et al.*, 1983; Jones and Cookson, 1983; Takatori *et al.*, 1994) while decreases of airborne microflora occurred during hot periods in some other reports (Filipello-Marchisio *et al.*, 1992; Mullius *et al.*, 1984).

Likewise, diurnal periodicities have been observed in fungal spores in previous studies: in 1991, Nus-

sbaum showed that fungal spore population was more abundant in the afternoons in arboreal microenvironments while some reports measured an increase of fungal spores during the night (Jones and Cookson, 1983), depending on species. Diurnal variation was not evident between 9.00 and 17.00 h in the present study. In Marseilles, this result could be due to confounding factors such as pollution or human activities, while in Porquerolles Island it could be explained by the great distance between fungal spore sources and the sampling site, inducing a dilution of fungal population. Nevertheless, early morning and late evening samples would have been necessary to evaluate the diurnal evolution of airborne microflora.

Airborne viable particles depended on weather parameters in Marseilles as well as in Porquerolles Island: wind velocity affected significantly bacteria counts, wind direction influenced significantly fungal spore counts, temperature influenced both bacteria and fungi counts, whereas no significant effect of relative humidity on airborne particles was evident. The influence of wind velocity as a dilution and survival factor of airborne bacteria and fungi has been largely demonstrated in dispersion models and environmental reports (Lightart and Kim, 1987; Pasanen *et al.*, 1992). In the present study, wind velocity induced an increase of bacteria counts in both urban and natural environments.

Wind direction influenced airborne bacteria and fungi in the two sampling sites, but only fungal spore counts were affected significantly. Airborne microflora increased when wind originated from NW and was at its lowest level when it originated from SE. Marseilles and Porquerolles Island are located in coastal areas, each sampling site was at 1 km North from the sea. These results suggested that wind originating from the South could submit airborne microorganisms to the well-known toxic effect of sea aerosols both in Marseilles and Porquerolles Island (Bovallius *et al.*, 1978; Rosas *et al.*, 1993).

Temperature was also a significant variation factor for airborne microorganisms in the two sampling sites: optimal temperature varied between 15 and 20°C. This result agreed with previous experimental reports where *E. coli* was shown to have a maximal survival at 15–20°C in the airborne state. Moreover, most environmental studies have reported the significant effect of temperature on bacteria and fungal spore counts depending on species and sampling environment (Bovallius *et al.*, 1978; Jones and Cookson, 1983; Rosas *et al.*, 1993).

Relative humidity had no significant effect on viable particles in Marseilles and in Porquerolles Island. This result conflicts with previous environmental reports where bacteria and fungi levels increased with relative humidity (Macher *et al.*, 1991; Paya-Vicent and Suarez-Fernandez, 1984), and with experimental studies concerning the viability of bacteria in the airborne state (Wathes *et al.*, 1986; Muller and Dinter, 1986). In the present study, correlation matrix of weather parameters showed that, contrary to wind velocity and temperature which were independent of the other meteorological parameters, the relative humidity was strongly correlated with wind direction ($P = 0.002$): it was high when wind direction varied between SW and SE. Thus, the absence of correlation between the relative humidity and viable airborne particles could be explained by the confounding effect of wind.

No correlation could be found between bacteria and fungi counts in the two sampling sites, confirming the fact that bacteria and fungi were not submitted to the same variation factors and illustrating the susceptibility of species to external factors of survival and dispersion.

Partial identification of bacteria showed that airborne contamination was composed of 60% Gram negative bacteria in Marseilles, whereas Gram positive bacteria were in the majority in Porquerolles Island. Increase of Gram negative bacteria in urban areas has been reported in previous studies (Chihara and Someya, 1989; Jones and Cookson, 1983; Macher *et al.*, 1991) suggesting that sampling environment had a qualitative influence on airborne contamination. In the present study, additional sources of contamination in sampling environment such as hospital incinerator which has previously been reported to produce bacteria in the airborne state (Allen *et al.*, 1989) may have increased the level of Gram negative bacteria at the sampling site. Consequences of high concentrations of airborne microorganisms for human health can be discussed. In the home environment, the role of airborne microorganisms as determinants of respiratory and general health has been well established: fungal spora and volatiles (alcohols, aldehydes, esters and hydrocarbons) have been shown to have irritant and allergenic properties while Gram negative bacteria producing endotoxins have been associated with respiratory diseases (Cox, 1989; Flannigan *et al.*, 1991; Gravesen, 1979). Natural outdoor sources of contamination are generally considered to make a major contribution to airborne microorganisms within buildings (Macher *et al.*, 1991). On this basis, although viable outdoor microorganisms are not air pollutants, they should be considered as a factor affecting air quality, more particularly in the vicinity of hospitals.

Acknowledgements—This work was supported by Centre d'Etudes du Bouchet (France). We wish to thank Labortoire LOPT (University of Toulon) for technical help in Porquerolles Island.

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