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Indoor air quality—bioaerosol measurements in domestic and office premises

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Abstract

Bioaerosols play a significant role in indoor air pollution as they can be pathogenic or cause an allergic reaction following inhalation. In this study, indoor bioaerosol measurements are presented from a typical apartment in Athens and other indoor establishments in Chania, Greece. Concentrations of airborne bacteria and fungi were measured as colony forming units per cubic meter of air (CFU/m^3) collected by impaction onto agar plates. Samples were taken by using a Merck MAS-100 bioaerosol collector. The bioaerosol data are presented together with indoor and outdoor PM_{10} and $PM_{2.5}$ particle measurements.

Keywords: Bioaerosols; Indoor; Airborne bacteria; Impactor; CFU

1. Introduction

Bioaerosols consist of all airborne particles of biological origin, i.e., bacteria, fungi, fungal spores, viruses, and pollen and their fragments, including various antigens. Particle sizes may range from aerodynamic diameters of ca. 0.5 to 100 μm (Nevalainen et al., 1991; Cox and Wathes, 1995). Airborne

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micro-organisms become non-viable and fragmented over time due to desiccation. Indoor air contains a complex mixture of (i) bioaerosols such as fungi, bacteria and allergens, and (ii) non-biological particles (e.g., dust, tobacco smoke, cooking-generated particles, motor vehicle exhaust particles and particles from thermal power plants). Exposure to several of these biological entities as well as microbial fragments, such as cell wall fragments and flagella, etc. and microbial metabolites e.g., endotoxin, mycotoxins and VOCs, may result in *adverse health effects*. In particular, increase in asthma attacks and bronchial hyperreactivity has been correlated to increased bioaerosol levels (Ross et al., 2000). Recent epidemiological studies (data from six US cities) suggest a strong relationship between mortality and source-specific PM concentrations (Lippmann et al., 2003).

Elevated levels of particulate air pollution have been associated with decreased lung function, increased respiratory symptoms such as cough, shortness of breath, wheezing and asthma attacks, as well as chronic obstructive pulmonary disease, cardiovascular diseases and lung cancer (World Health Organisation, 2002). More than 80 genera of fungi have been associated with symptoms of respiratory tract allergies (Horner et al., 1995). *Cladosporium*, *Alternaria*, *Aspergillus* and *Fusarium* are amongst the most common allergenic genera. Metabolites of fungi are also believed to irritate the respiratory system. Furthermore, non-biological particles may serve as carriers of fungal allergen molecules into the lung independently of the whole fungal spore. In the case of combustion particles such as tobacco, smoke or cooking-generated particles, such an interaction could have serious implications. Allergenic molecules could conceivably be carried into the lung at a greater depth than a fungal spore would be expected to penetrate (Lippmann et al., 2003).

In non-industrial indoor environments, the most important source of airborne bacteria is the presence of humans (Stetzenbach, 1997). In particular activities like talking, sneezing, coughing, walking, washing and toilet flushing can generate airborne biological particulate matter. Food stuffs, house plants and flower pots, house dust, pets and their bedding, textiles, carpets, wood material and furniture stuffing, occasionally release spores of *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Penicillium*, *Scopulariopsis* into the air (Cox and Wathes, 1995; Maeir et al., 2002).

Although indoor environments are considered to be protective, they can become contaminated with particles that present different and sometimes more serious risks than those related to outdoor exposures, when their concentrations exceed recommended maximum limits. These are: 1000 CFUs/m^3 for total number of bioaerosol particles set by the National Institute of Occupational Safety and Health (NIOSH), 1000 CFUs/m^3 set by the American Conference of Governmental Industrial Hygienists (ACGIH) with the culturable count for total bacteria not to exceed 500 CFUs/m^3 (Cox and Wathes, 1995; Jensen and Schafer, 1998).

In this study, indoor bioaerosol measurements are presented from a typical apartment in Athens and other indoor establishments in Chania (Crete, Greece) in an effort to characterize the level of bioaerosols encountered in typical indoor environments in Greece and see whether maximum bioaerosol levels are exceeded.

2. Materials and methods

2.1. Study sites and sampling location

2.1.1. Pagrati campaign

Indoor bioaerosol measurements were taken in a 3rd floor typical one bedroom apartment of a 10 year old building with a kitchen and living room, located in Pagrati which is within the densely populated

metropolitan area of Athens. The apartment was occupied by one person. The apartment is located close to a major traffic route, Formionos St. Bioaerosol measurements were taken on 2/4/2002, 3/4/2002 and on 29/8/2002 during the morning and early afternoon period. Outdoor bioaerosol and PM measurements were also taken at the front balcony of the apartment and during the sampling periods the number of the individuals present was also recorded. PM measurements were taken continuously during the campaign period.

2.1.2. Chania campaign

In order to examine the effect of long-term differences in weather conditions and the contribution of the people working in the sampling location on the bioaerosol formation, seven sampling campaigns were conducted with the following dates: 24/09/2003, 30/09/2003, 03/10/2003, 14/10/2003, 17/10/2003, 18/11/2003 and 02/12/2003. Approximate meteorological conditions prevailing in the study area were also recorded. This campaign took place in an office building, which is located in the centre of the city of Chania (Crete, Greece).

The building was built in 1895 with the later addition of a third floor where the bioaerosol measurements were made. The particular office (Office I) that was chosen for sampling was the largest one on the floor (33.4 m^2) and had two windows. There are five staff members working in the office that accepts many visitors throughout the day. For comparison purposes, measurements were also made in another office (Office II), the smallest one (21 m^2) on the same floor, which had also two windows and four staff members. In addition, measurements were also taken in the high traffic corridor in front of Office I on the 3rd floor. At all times, people were waiting in the corridor to enter the various offices on the floor. The periods of direct sunlight were minimal because of the northern orientation of building. This increases the probability of survival of airborne microorganisms, as the sun's direct UV radiation can reduce the viability of many microorganisms (Peccia et al., 2001). The building has a central air conditioning system that was not in use during the whole campaign.

The primary goal of the bioaerosol sampling was the quantitative evaluation of the viable airborne bacteria and fungi. Besides the standard enumeration of culturable microbes as CFU/m^3, we attempted to identify and evaluate the colonies through their specific colour, turbidity or other characteristics that appear when grown on selective media. The qualitative assessment was based on the characteristics given in Merck's "Microbiology Manual 2000''.

For each date, all the bioaerosol samples were taken during working hours. At each sampling location within the building, two duplicate samples were collected, for each of the four media and for three different sample volumes (200, 400 and 800 L). During the sampling time, the number of the individuals present was also recorded.

2.2. PM sampling

The mass concentration of ambient aerosol (particulate matter) was measured over 24 h periods, indoors and outdoors for the two size fractions PM_{10} and $PM_{2.5}$. Indoor sampling was made using a cascade impactor (Dekati) with fractioning intervals at 10 and 2.5 μm at a constant flow rate of 29 L/min. Sampling outdoors was performed by two separate custom made sampling heads with fractioning intervals at 10 and 2.1 μm. The PM_{10} head was designed according to the European low volume PM_{10} reference sampler (CEN, 1998). The mass concentration was determined gravimetrically by weighing the filters/collection substrates before and after sampling on a 10^{-5} g resolution balance (Sartorius, B210). Samples were left

to equilibrate for 48 h in the weighing room, which has minimal variation in humidity and temperature (RH $63 \pm 2.5\%$ and temperature $24 \pm 2.0\,^{\circ}C$). Relative humidity was considered to have minor influence on the gravimetric results. The total uncertainty of the mass concentration was calculated to an average of 5.3%. The standard error of the weighing procedure was determined from repeated weighing of blank substrates and by combining with the uncertainty of the air volumes sampled.

2.3. Bioaerosol sampling methods

The sampling device used was the MAS-100 air sampler (a multi-jet impactor plate with 400 holes operating at a flow rate of 100 L/min), based upon the principles described by Andersen (Buttner et al., 1997; Solomon, 2003) with a high-performance suction pump with continuous monitoring of the airflow. The resulting air-stream is directed onto an agar surface in a standard Petri dish (diameter 90 mm). This system measures the incoming air flow, it regulates the aspirated air to a constant flow of 100 L/min and can collect up to 1000 L per run. The impaction speed of the airborne microorganisms on to the agar surface is approximately 11 m/s which corresponds to stage 5 in the typical six-stage Andersen-impactor (Merck, 1999).

2.4. Methods of analysis

Analysis was carried out using simple colony enumeration on incubated 90 mm Petri dishes containing modern rapid agar systems. Agar was prepared and sterilized and subsequently spread onto the Petri dishes. After collection, the plates were incubated at 30 °C for 36–48 h and the colonies were counted. Results are expressed in colony forming units per cubic meter of air (CFU/m^3).

2.4.1. Media

The employed media were the following four suggested by the equipment manufacturer (Merck, 2000):

1. *Endo agar*: Selective culture medium for the detection and isolation of *E. Coli* and *coliform* bacteria in various materials.
2. *Peptone yeast agar*: Selective culture medium for total bacteria (Pepper et al., 1995).
3. *MacConkey agar*: Selective agar for the isolation of gram-negative microorganisms e.g., *Salmonella*, *Shigella* and *Coliform* bacteria.
4. *Levine-EMB agar* (Eosin Methylene-blue Lactose Agar acc. to Levine): Selective agar for the isolation and differentiation of *E. coli* and *Enterobacter* and for the rapid identification of *Candida albicans*.

3. Results and discussion

3.1. Variability of CFU counts

The CFUs enumerated on the Petri dish are related to the bioaerosol level in the ambient air by the simple relationship

$$[CFU/m^3] = [CFU]_{Petri} \times (1000/V_S),$$

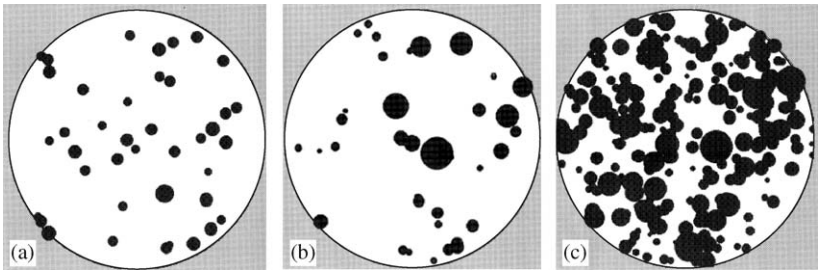

Fig. 1. Schematic diagram of CFU counts on a Petri dish: (a) and (b) variability due to low CFU count on the dish, and (c) increased masking effect due to high number of CFUs on the dish.

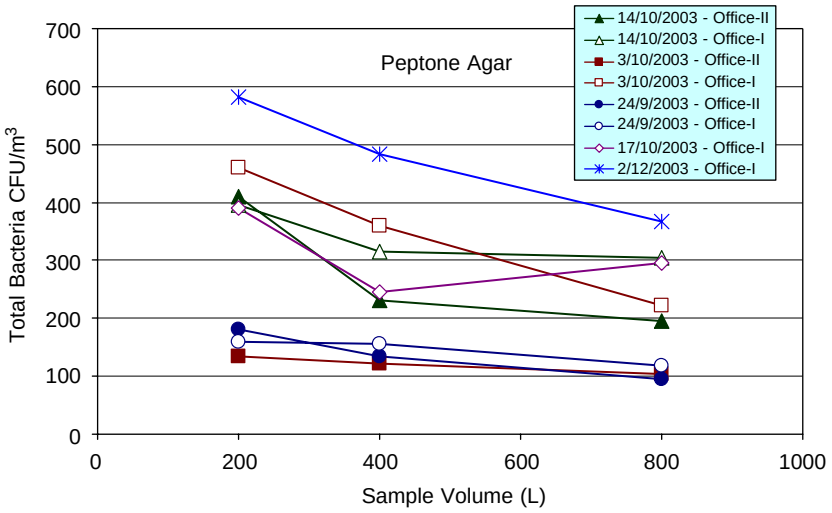

Fig. 2. Data from the Chania campaign indicating a general trend of colony masking at higher volumes when the CFU count reaches 200.

where V_S is the volume in litres passed through the bioaerosol sampler. Typically V_S is in the range 50 to 1000 L for the MAS-100 sampler which implies that the factor $(1000/V_S)$ can take values from 1 to 20. As a result, the standard error in $[CFU]_{Petri}$ is amplified by a factor between 1 and 20 depending on the size of the volume sampled. Hence, optimum measurements should theoretically be obtained at higher sample volumes; however, when the CFU count on the Petri dish increases to approximately 200 units, the masking effect becomes significant. In this case, some of the colonies are not enumerated due to colony overlapping (Fig. 1).

As a result of the above observations, higher sample volumes are expected to provide more reliable estimates of the bioaerosol concentration in the air provided that the total number of CFU enumerated do not exceed 200. Otherwise, one should trust more the estimates obtained with smaller sample volumes. This general trend is verified by the data collected in the two offices in Chania as shown in Fig. 2. As one

Table 1
Mean daily values of PM measurements

Date	Outdoors ($\mu g/m^3$)		Indoors ($\mu g/m^3$)	
	$PM_{2.5}$	PM_{10}	$PM_{2.5}$	PM_{10}
01/04	27	30	16	20
02/04	44	56	27	28
03/04	28	36	17	22
28/08	40	62	34	42
29/08	61	74	35	38
30/08	—	37	20	22

is not aware ahead of time what the expected bioaerosol concentration in the air might be, it is prudent to obtain estimates at different sample volumes (e.g., 200, 400 and 800 L). The viability of the collected microbes on the Petri dish is not expected to be affected by the increased sampling period; however, this is not the case when the collection is carried out with other devices, e.g., membrane filters, all-glass liquid impingers with mixtures of glycerol–water as collection liquid, etc. (Buttner et al., 1997).

3.2. Pagrati campaign

The mass concentration of PM indoors and outdoors is given in Table 1 during a 3 day period (24 h averaged) centered around the day of bioaerosol sampling. Parallel PM measurements were available for two out of the three bioaerosol sampling campaigns and are given in Table 1. During the Pangrati campaign, a total of 14 samples of 24 h sampling duration were collected at the residence under study. PM_{10} outdoor mass concentrations ranged from a minimum of 24 $\mu g/m^3$ to a maximum of 128 $\mu g/m^3$ with a mean of 52.5 $\mu g/m^3$. As seen from Table 1, the outdoor PM concentration on the 2nd of April is enhanced, with a concurrent moderate increase indoors. In general, measurements show that indoor concentration is mainly driven by infiltration of outdoor aerosol. This observation is supported by data derived from a longer 2 weeks measurement period (Eleftheriadis et al., 2003). In the absence of indoor aerosol sources e.g. smoking, cooking the indoor concentration is lower than outdoors. Infiltrating aerosol from outdoors is depleted in its ultrafine (0.001–0.1 μm in diameter) and coarse (1–100 μm) fraction mainly due to indoor deposition (Nazaroff, 2004; Monn, 2001). The latter has a pronounced impact on mass concentration as observed in the current measurements. It is interesting to note that as bioaerosol counts (CFU/m^3) are increasing, PM mass exhibits a similar behavior (Parat et al., 1999); however, in the same study it was reported that no correlation was found between different experiments (i.e., at different environmental conditions and sources). Nonetheless, combining particle with bioaerosol counting may help to evaluate individual exposure to airborne bacteria, by detecting instantaneously rapid variations which could be undetected by periodic microbial measurements (Parat et al., 1999). In a more recent study, Hargreaves et al. (2003) examined the relationship between indoor airborne fungal and non-biological particle concentrations in 14 residential houses in Brisbane, Australia and showed that no statistically significant associations were found between the fungal spore and sub-micrometer particle concentrations or $PM_{2.5}$.

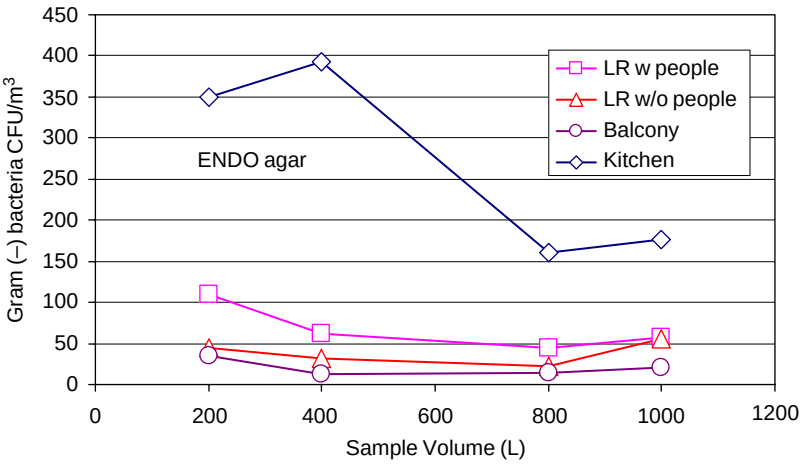

Fig. 3. Bioaerosol measurements on 3/4/2002. Comparison of bioaerosol levels in different rooms.

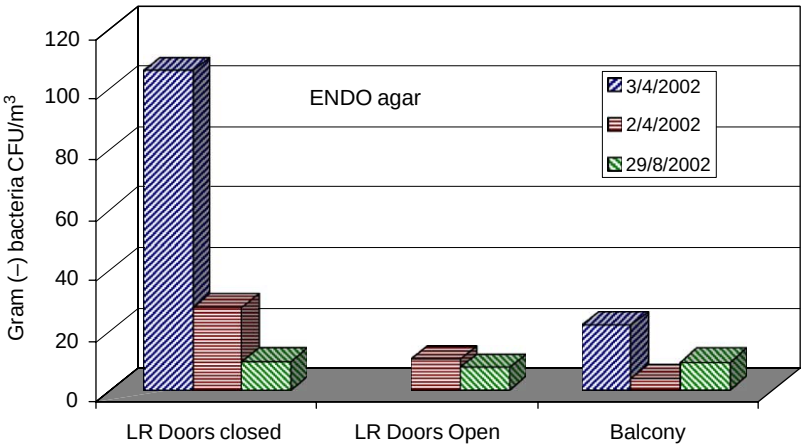

Fig. 4. Effect of air exchange on Living Room bioaerosol measurements and comparison with outdoor bioaerosol levels (reported values are averages from 200, 400 and 800 L sampling volumes).

In Fig. 3, the bioaerosol counts are compared in different rooms of the Pagrati apartment. The level of bioaerosols in the kitchen area is significantly higher compared to any other rooms or to outdoor bioaerosol levels on the balcony. The profile of the CFU versus sampled air volume for the kitchen confirms the need to sample a range of volumes, as CFU counts approaching 200 may have a significant masking effect. Obviously, the kitchen had a higher bioaerosol count as old food stuff was around, dishes was not regularly washed and general state of cleanness was rather poor as the residing student was always too busy. . .

In Fig. 4, the effect of air exchange with the outdoors is presented for the Pagrati apartment during different days. As seen, the CFU counts in the living room are higher when the doors are closed to

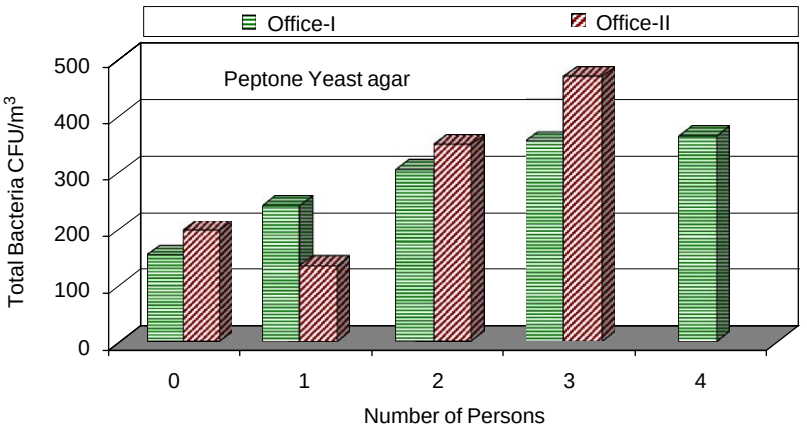

Fig. 5. Effect of human presence on indoor bioaerosol measurements.

those found with intermittent opening of the balcony doors for periods of 15 min. In addition, the indoor bioaerosol measurements are consistently higher that the outdoor level. When the bioaerosol and PM measurements were performed concurrently, the increased PM_{10} concentrations outdoors, and to some extent indoors, do not result in a similar bacterial count trend. This indicates that PM mass does not provide information on likely bioaerosol exposure.

3.3. Chania campaign

During the measurement campaign in Chania, several samples were taken under different conditions to study the effect of potentially significant environmental and anthropogenic factors. In Fig. 5, the effect of human presence on bioaerosol counts is shown for both offices. As seen, there is rather systematic increase in the CFU count as the number of persons in each room increases. Of course, this is not an unexpected observation as the presence of humans is considered the most significant source of bioaerosols in indoor environments (Stetzenbach, 1997).

In Fig. 6, the bioaerosol counts are compared between Office I and the high traffic corridor on the same floor. As seen, there is no significant difference between the total bacterial counts between the two different sampling areas. In both locations and during all measurements, there were at least three persons present. On the other hand, there appears to be a noticeable difference in Gram-negative bacterial counts obtained by CFU measurements from the Corridor on ENDO agar for all volumes sampled. A plausible explanation could be the different ventilation between the two areas.

In Fig. 7, the bioaerosol counts are compared for the same office on three different days where the meteorological conditions were different. As seen, the effect of the meteorological conditions on the total bacterial counts is rather minimal whereas there is a noticeable difference between the CFU of Gram-negative bacteria. It appears that the number of Gram-negative bacteria increases as the temperature decreases and the relative humidity increases in a generally warm environment. This observation was also confirmed on several other occasions (data not shown).

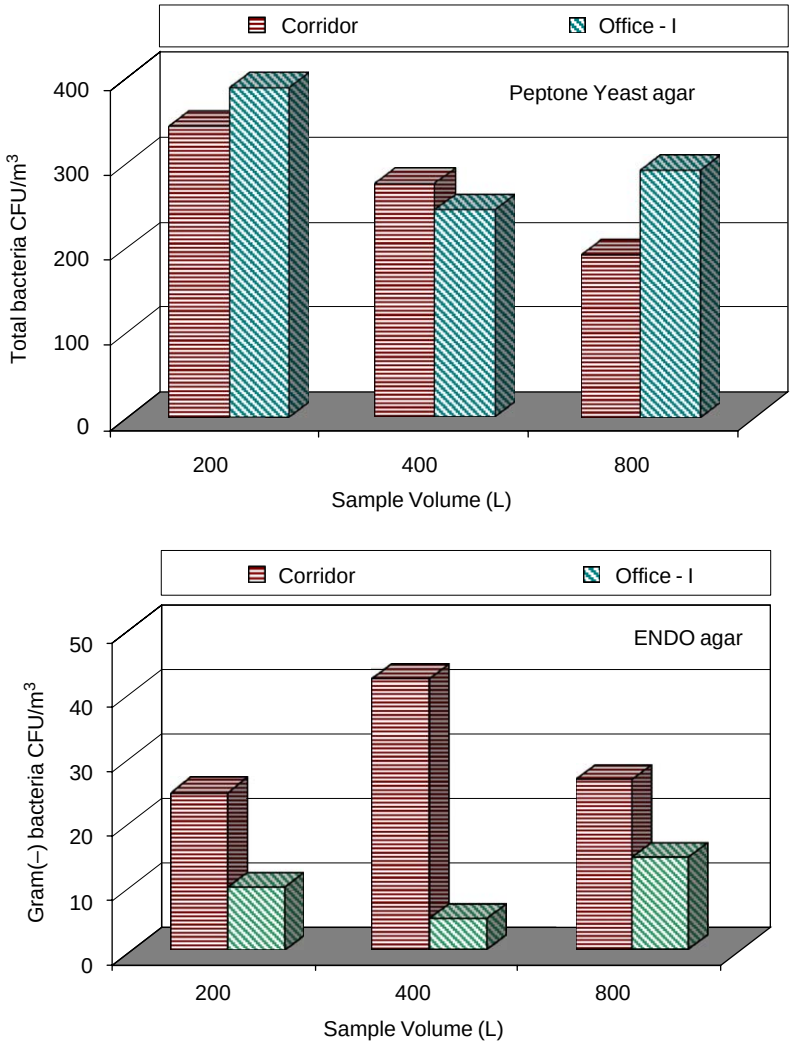

Fig. 6. Comparison of bioaerosol measurements between Office-I and the high traffic Corridor using (a) peptone yeast agar and (b) ENDO agar.

3.4. *Effectiveness of agar*

ENDO agar and *Peptone yeast* agar was used during all the measurements. Microbial growth on both media was very good. *MacConkey* agar used only at the first three sampling campaigns and *Levine Agar* only on 14/10/2003. Unfortunately the response of the last two media was rather poor, namely, very few colonies per dish (typically less than 5) even at high sampling volumes were seen. The *Levine* Agar exhibited only mold growth.

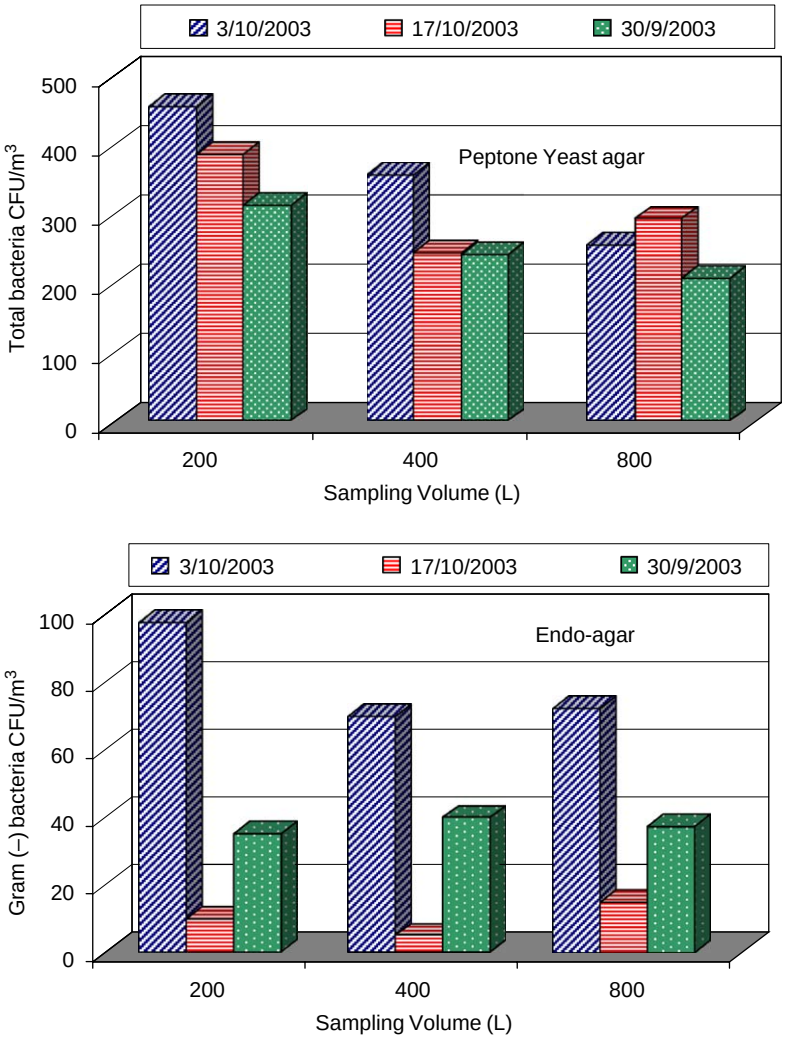

Fig. 7. Effect of environmental conditions on bioaerosol measurements in Office-I using (a) peptone yeast agar and (b) ENDO agar (30/9/2003: RH = 62%, $T = 26\,^{\circ}C$, no rain; 3/10/2003: RH = 60%, $T = 27\,^{\circ}C$, no rain; 17/10/2003: RH = 72%, $T = 19\,^{\circ}C$ rained earlier).

4. Conclusions

- Bioaerosol counts are an integral part of the indoor pollution characterization puzzle and important for quantification of airborne allergens and pathogens.
- In the Athens and Chania apartments, only one bioaerosol count of total bacteria was found to exceed $500\,CFU/m^3$, the guideline set by the World Health Organization (WHO, 1990, 2002).
- The presence of people is the most significant parameter resulting in elevated indoor bioaerosol counts in the absence of significant indoor or outdoor sources.

- The tested areas were not close to known outdoor bioaerosol sources, e.g., parks, landfills, etc. The humidity inside the homes never reached excessively high values. Outside bioaerosol counts were always lower than indoors, suggesting the presence of indoor sources. Room ventilation by opening a window for a period of time when the outdoor PM mass is near the daily minimum would reduce the presence of bioaerosols.

Acknowledgements

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