

Spectrophotometric analysis as a complementary technique to aerobiology in the study of solid particles in the air

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Received 25 November 1996; received in revised form 6 October 1997; accepted 12 March 1998

Abstract

Hitherto, aerobiology has mainly focused on studying and analysing the biologic material found in the atmosphere (pollen grains and fungal spores), due to its allergologic and agricultural interest. However, the increase in respiratory tract problems caused by other solid particles suspended in the air has made studies including biologic and non-biologic material more frequent. This paper describes a simple technique based on spectrophotometric analysis. The results thus obtained can complement the outcome obtained by traditional aerobiologic techniques if the method is used properly and the equipment properly calibrated and an idea of the solid particles in the air can be obtained. The combination of both aerobiological and spectrophotometric analysis makes it possible to estimate the concentration of solid-particle material in the air, and we can also estimate the percentage of pollen grains and fungal spores among the general spectrum of particles. The results also show that a great part of the solid particulates originate from human activities. However, it was also observed that they emerge from natural sources as such as fungi and higher plants. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Aerobiology; Solid particles; Spectrophotometer

1. Introduction

In samples obtained using a Hirst-type suction volumetric sampler, a great amount of dust, sand, ash, remains of hydrocarbons and soot is frequently found, besides pollen grains and fungal spores. These particles come not only from natural sources but also from sources derived from human activity.

Many studies have shown that the presence of these particles contribute to an increase in respiratory tract problems either as agents that cause illnesses in themselves (Kagamimori et al., 1986; Muranaka et al., 1986; Magnussen et al., 1993; D'Amato et al., 1994) or as the adjuvant effect that is provoked in people who suffer from respiratory allergies (Ishizaki et al., 1987;

Berciano et al., 1989; Morrow Brown, 1991; Santra et al., 1991; Dominguez et al., 1993).

In some papers, the methodology employed to analyse solid-particle material in air only allows differentiation of material on its size and not its nature (Lebowitz et al., 1992). In the same way, it is rare to find studies on non-biotic particles in aerobiology, although some attempts have been made to analyse them. It is worth mentioning the works of Mäkinen (1978), Leuschner and Boehm (1981) and Boehm and Leuschner (1989).

In this research work, aerobiologic techniques and spectrophotometric techniques have been used. These have enabled us to obtain, on the one hand, a quantitative analysis of the existing solid particles in the samples, and, on the other hand, a study of the percentage of representativity that the different groups of particles have, regarding the general spectrum of solid particles in the air.

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2. Materials and methods

Aerobiologic samples were obtained during 1990 and 1991 from a suction Hirst-type volumetric sampler (Burkard spore-trap, Burkard Manufacturing) which was placed on the flat roof of the Faculty of Science at the University of Cordoba (4°45'W, 37°50'N). This device is quite efficient for collecting particles measuring between 2 and 100 μm , a range that includes both biotic material such as pollen grains and several types of fungal spores, and non-biotic material. However, the efficiency for particles below 2 μm is lower than other samplers (Emberlin and Baboonian, 1995).

Optical microscopy has been used to analyse qualitatively the solid particles in the sample. In this analysis we have differentiated the biotic content of the air coming from natural sources (higher plants and fungi) from those originating from other sources, some of them natural and others derived from human activity. The biotic material has been quantified using the method recommended by the Spanish Aerobiology Network (REA) (Dominguez et al., 1992) and classified as pollen grains and fungal spores. Regarding the non-biotic material, we have considered as a whole the total amount of inert particles left, among which we have distinguished different components such as dust, ash, soot, hydrocarbons and partially-burnt plants.

In order to estimate the concentration in which solid material appears in the samples and, therefore, in the air, an absorbance spectrophotometer with a gel-scan function has been used, Beckman-DU7 (Beckman, Palo Alto, CA), following an analogous technique to that described by Leuschner and Boehm (1981) and Boehm and Leuschner (1989). We have employed a light absorbance spectrophotometer instead of a photometric kit for electropherograms.

The parameters used for the photometric scanning of the samples were as follows: number of readings per mm of sample = 20; The source of light used was visible light—400 nm wavelength. Before any photometric scanning, the spectrophotometer was calibrated using as standard a microscopic slide with a fragment of the sticky tape used for trapping the particles which had not been exposed. In this way, when we scan the samples, every particle on the tape can be measured depending on its light absorbance as a function of this reference standard. The spectrophotometric scanning produces a graphic image with known rate per hour according to a scale. Thus, the correlation between data on pollen grains and fungal spores becomes possible.

In order to differentiate the proportion of biotic particles among the complete spectrum of solid particles, we have made an analysis of Pearson's correlation between the spectrophotometric rates of absorbance and both groups of biotic particles, as well as the total amount, for both monthly and seasonal data.

3. Results

The results of pollen grains/ m^3 , fungal spores/ m^3 , and the absorbance percentage after the photometric scanning of the samples, are shown in Fig. 1.

The highest concentration of pollen is recorded in spring, when the percentage reached 64% of the annual total in 1990 and 82% of the annual total in 1991. The highest concentration for the last 15 years regarding pollen from the main taxa of spring bloom (*Olea*, *Poaceae*, *Quercus*, *Platanus* and *Morus*) (Dominguez et al., 1993) was measured in 1991.

The spores show only one peak during autumn 1990, and two peaks, one in autumn and the other in spring 1991; the shape of the curve is possibly related to the meteorological conditions of the relevant years. It is also worth mentioning that out of the total biotic particles analysed, spores reached 95.5% of the annual total in 1990, and 88% of the

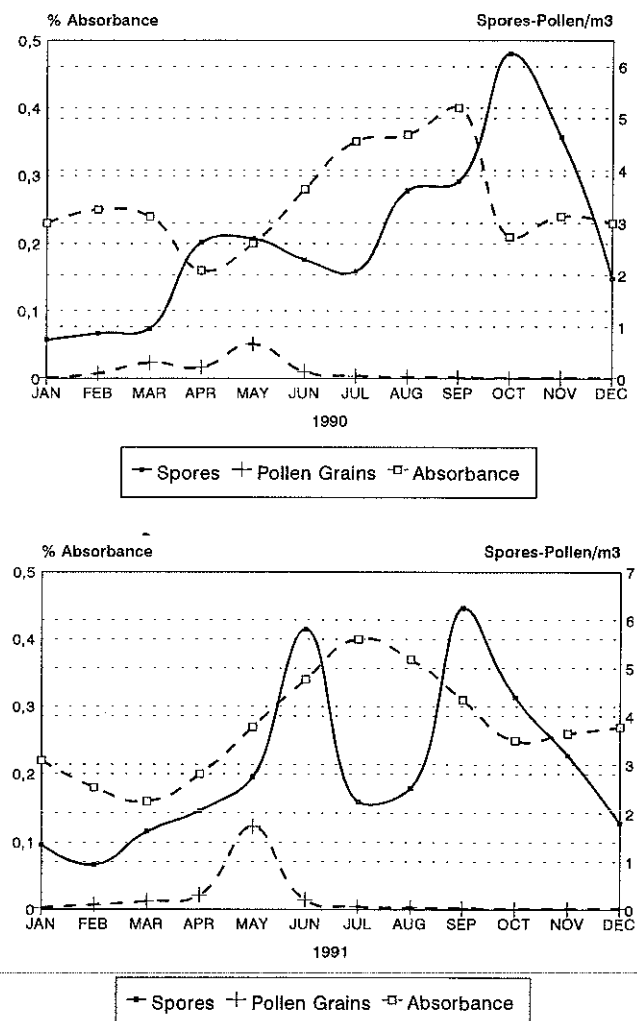


Fig. 1. Yearly variation of spectrophotometric absorbance, fungal spores and pollen grains, 1990 and 1991.

Table 1

Pearson correlation analysis between spectrophotometric absorbance, total biotic particles, fungal spores and pollen grains; applied for the whole year

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1990												
Spec-totals			**	*	**					**		
Spec-spores			**	*	**					**		
Spec-pollen				**	**	*	**					
1991												
Spec-totals				*								
Spec-spores				*								**
Spec-pollen			**	**	**							

Significance levels: * 95%; ** 99.9%.

annual total in 1991. Due to this high contribution, the statistical results obtained for total biotic particles do not differ from those obtained for fungal spores.

The pattern of spectrophotometric absorbance is fairly similar, with an annual mean of 0.26 in both years, the highest peaks being recorded during summer months. These results coincide with those obtained by Ali-Mohamed (1991) in his work on inorganic particles in the air of Isa Town, Bahrein.

Table 1 shows the results obtained after Pearson's correlation analysis which allows us to know the approximate contribution that biotic particles make to the general spectrum of solid particles. In 1990, the level of significance is 99.9% regarding pollen grains and fungal spores, as well as the total of both, only during May. A similar correlation is valid for spores only during March and October, but negative for pollen grains in April and July. A significant correlation, of 95%, was found with spores in April and with pollen grains in June.

In 1991, the statistical correlation is significant for all groups considered in April. However, while the correlation between photometric absorbance and pollen grains is significant at 99.5%, it is only at 95% between spores and photometric absorbance. During March and May, a significant correlation of 99.9% was reached with pollen and in December, the percentage of significance was the same with spores.

The results obtained after the application of the same parametric test to the seasonal data are shown in Table 2. The highest correlation between the rates of spectrophotometric absorbance, the total amount of biotic particles, fungal spores and pollen grains, was recorded in spring in both years. In the case of pollen grains, a 99.9% correlation was reached during summer 1991. For spores however, a similar correlation but at a lower level of significance could be noted in autumn and winter 1990.

4. Discussion

The results reveal that the contribution of biotic particles to the spectrum of solid particles in the air is particularly relevant at specific periods of time during the year. After the application of the statistical tests, certain correlations with an acceptable level of significance were observed in situations in which low absorbance rates coincide with high concentrations of pollen grains and fungal spores.

A significant correlation of 99.9% was obtained in spring 1990 for all types of particles considered. A lower percentage (95%) was recorded with the group of spores, in autumn as well as in winter (Table 2). Fig. 1 demonstrates that in every previous correlation, the fall in the curve of spectrophotometric rates coincides with the rise in the curves of concentrations of pollen and spores. From the results of the monthly data (Table 1), a significant correlation in the same situation may be confirmed: from March to July, with a maximum in May, when the significance level is 99.5% for all groups, and again in October, but only for fungal spores.

The results of the following year (1991) are similar. Considering the seasonal data, it is again in spring when the correlation is significant for both the spectrophotometric absorbance rates and the biotic particles. Besides, pollen grains showed a significant correlation during summer, probably because the flowering of some spring plants lasted until the beginning of summer; this could be the reason for high pollen rates during this season. Fig. 1 shows the fall of the absorbance curves coinciding again with the rise of pollen and spore concentrations. The monthly results confirm the existing correlation with pollen in the period of higher pollen presence in the air and with fungal spores in December. This last result is not shown in the seasonal data as a consequence of the absence of correlation during the remaining months of the season.

Table 2

Correlation analysis between spectrophotometric absorbance, total biotic particles, fungal spores and pollen grains; applied for seasonal data

	Winter		Spring		Summer		Autumn	
	1990	1991	1990	1991	1990	1991	1990	1991
Spec-totals			**	**			*	
Spec-spores	*		**	**			*	
Spec-pollen			**	**	**	**		

Significance levels: * 95%; ** 99.9%.

It may be concluded that there is a significant correlation between the rates of spectrophotometric absorbance and biotic particles during the periods of the year in which the absorbance rates are not very high, because the air at that time is free from pollutant particles of non-natural origin and, on the contrary, the concentration of pollen grains and fungal spores is very high. In other words, the results obtained after the spectrophotometric scanning of the samples corresponds, in these situations, with existing concentrations of biotic material. However, in the periods of the year in which solid non-biotic material is more abundant, as atmospheric conditions allow its concentration, the difference between biotic particles and the rest of the particles composing the spectrum could not be recorded.

The methodology used for spectrophotometric analysis is based on the amount of light that can be absorbed by the particles, which is higher in those with a dark colour: e.g. hydrocarbons, dust, soot. The light colours of pollen grains and the small size of several types of spores, do not help light absorption and its projection in the graph obtained after the scanning, corresponds with minimum peaks. This explains why a very high contribution to the spectrum from the biotic particles can be noted when the air is clean from non-biotic pollutants, as these can produce a screening effect on pollen grains and fungal spores. Furthermore, in the case of fungal spores, the commonest types of the area should be taken into account, depending on whether these are one type or the other, thus finding a higher or lower rate in the spectrum of solid particles in the air. Therefore, in the study area, *Cladosporium* is one of the most abundant species in the air (Mediavilla, 1995). Two species of *Cladosporium* can be identified, *C. cladosporioides* and *C. herbarum*, the former having a transparent colour and a size of $3-15 \times 2-6 \mu\text{m}$; the latter a dark colour and a size of $8-25 \times 4-8 \mu\text{m}$, both having a different level of light absorption. Consequently, they show a positive or negative rate in the percentage of spectrophotometric absorbance obtained.

Therefore, the spectrophotometric analysis of the samples carried out using a volumetric sampler is a reliable method to determine the concentration of biotic and non-biotic solid particles in the air, allowing in some cases the distinction of the particles according to their nature. It is advisable, however, to use a combined aerobiological and spectrophotometric methodology, taking into account the amount of biotic particles found during certain periods of the year, and its consequences in patients with allergy problems, caused by pollen grains and fungal spores.

Acknowledgements

The authors are grateful to the Spanish DGCyT for financial support granted through Project PB-92-0814-CO4-01. We would like to thank the Molecular Biology Department (University of Córdoba) for making examinations with the absorbance spectrophotometer possible.

References

- Ali-Mohamed AY. Estimation of inorganic particulate matter in the atmosphere of Isa Town, Bahrein, by dry deposition. *Atmos Environ* 1991;25(3):397–405.
- Berciano FA, Dominguez J, Alvarez FV. Influence of air pollution on extrinsic asthma. *Ann Allergy* 1989;62(2):135–41.
- Boehm G, Leuschner RM. Les polluants aériens a diverges altitudes. *Rev Int Pédiatrie* 1989;193:23–33.
- D'Amato G, Liccardi G, Cazzola M. Environment and development of respiratory allergy: I. Outdoors. *Monaldi Arch Chest Dis* 1994;49:406–11.
- Dominguez E, Galán C, Villamandos F, Infante F. Manejo y Evaluación de los datos obtenidos en los muestreos aerobiológicos. Dpto. Biología Vegetal y Ecología. Unidad de Monitorizaje Aerobiológico de la Universidad de Córdoba. Monografía REA/EAN 1992;1:1–13.
- Dominguez E, Galán C, Guerra F, Villamandos de la Torre F, Infante F, Mediavilla A. Spring pollen and related allergies in Southern Spain. *J Invest Allergol Clin Immunol* 1993;3(15): 271–5.

- Emberlin JC, Baboonian C. The development of a new method of sampling airborne particles for immunological analysis. In: Basomba A, Hernandez F de Rojas MD, editors. XVI European Congress of Allergology and Clinical Immunology, 1995:39–45.
- Ishizaki Y, Koizumi K, Ikemori R, Ishiyama Y, Kushibiki E. Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. *Ann Allergy* 1987;58(4):265–70.
- Kagamimori S, Katoh T, Naruse Y, Watanabe M, Kasuya M, Shinkai J, Kawano S. The changing prevalence of respiratory symptoms in atopic children in response to air pollution. *Clin Allergy* 1986;16:71–5.
- Lebowitz MD, Quackenboss JJ, Kryzanowski M, O'Rourke MK. Multipollutants exposures and health responses to particulate matter. *Arch Environ Health* 1992;47(1):71–5.
- Leuschner RM, Boehm G. Pollen and inorganic particles in the air of climatically very different places in Switzerland. *Grana* 1981;20:161–7.
- Magnussen H, Jorres R, Nowak D. Effect of air pollution on the prevalence of asthma and allergy: lessons from the German reunification. *Thorax* 1993;48:879–81.
- Mäkinen Y. Quantification of Dust in Aerobiological Slides. Aerobiology Laboratory, Department of Botany, University of Turku, Turku, Finland, 1978.
- Mediavilla A. Modelos de variación intradiurna y estacional de la concentración en la atmósfera del Género *Cladosporium*. Tesis Doctoral. Universidad de Córdoba, 1995.
- Morrow Brown H. Mobile slit for pollen and spores sampling on motorways. *Aerobiologia* 1991;7:69–72.
- Muranaka M, Suzuki S, Koizumi K, Takafuji S, Miyamoto T, Ikemori R, Tokiwa H. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. *J Allergy Clin Immunol* 1986;77(4):616–23.
- Santra SC, Gupta S, Chanda S. Air pollutants and aeroallergens interaction. *Grana* 1991;30:63–6.

