

Preliminary assessment of protein associated with airborne particles in Mexico City

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Abstract

The protein associated with airborne particles was measured during 1991 as an indicator of airborne biological material in different outdoor urban environments. Fifty air samples were collected simultaneously at three sampling sites, located in the north, south and downtown Mexico City, using a PM10 high-volume sampler (particles < 10 μm). The air filters were weighed and protein extracted using a phosphate buffer. Protein concentrations were determined by Lowry assay. The extracts were also analysed by SDS electrophoresis and IEF using a Phastsystem. High concentrations of airborne particles were recorded at the sampling sites with a geometric mean of 70.2 $\mu\text{g}/\text{m}^3$ in the south (residential area), 95.5 $\mu\text{g}/\text{m}^3$ in the center (urban-commercial area), and with the highest value of 108.9 $\mu\text{g}/\text{m}^3$ in the north (urban-industrial area). No statistically significant difference ($P > 0.05$) was observed among the protein concentrations from the sampling sites and the concentrations ranged from non-detectable to 2.54 $\mu\text{g}/\text{m}^3$. However, the protein concentrations presented significant difference ($P < 0.05$) with respect to rainy and dry seasons. The Spearman correlation coefficient between protein concentration and airborne particles concentration was statistically significant ($r = 0.50$). The molecular weights (MW) and isoelectric points (pI) for the proteins present in some of the extracts were determined. The values ranged from approximately 8000 to 106 000 Da and the pI values from nearly 4.0 to 5.85. This is important because the major allergens from inhalants are mostly acidic proteins with molecular weights in the range of 20 000–40 000 Da.

Keywords: Airborne particles; Protein; Aeroallergens; Mexico

1. Introduction

The motivation for evaluating particle matter in sizes $\leq 10 \mu\text{m}$, has been to examine the risk of potential adverse health effects from toxic or pathogenic particles small enough to penetrate the thoracic region (Solomon et al., 1989; Pope, 1991; Salazar et al., 1992).

Much work has been done on the characterization of airborne material, either viable or non-viable, in order to study the etiology of the most common allergenic reactions of the respiratory tract. In general, the incidence of aeroallergies has been related to exposure to pollen, spores, mite excreta, danders and dust, which are

frequently found in the environment (Hyde, 1973; Tovey et al., 1981; Chapman, 1986; O'Rourke et al., 1989; Hamilton et al., 1992). In addition, their allergenic activity has been correlated with protein, glycoprotein and/or carbohydrate content (Yunginger et al., 1980). These allergens usually have a relative molecular mass with an upper limit of approximately 50 000 Da, probably determined by the reduced ability of substances of higher molecular weight to permeate the mucosal membranes of the respiratory tract (Schneeberger, 1974).

In Mexico City, with approximately 15 million inhabitants, the urbanization and industrial processes have produced alterations such as reduction of the green areas, soil pollution (Rapoport et al., 1983), modifications of the weather conditions (Oke and Zeuner, 1992), and an increase in the air pollution levels (Calderón-Garcidueñas et al., 1992; Báez et al., 1993). This study was undertaken to determine the seasonal and spatial variations of the protein concentrations extracted from

Abbreviations: IEF, isoelectric focusing; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; kDa, kiloDaltons; MW, molecular weight; pI, isoelectric point.

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air filters, as a possible indicator of airborne biological material either viable or non-viable. Moreover their molecular weight and the isoelectric point were determined in order to identify some chemical properties of the potential aeroallergens.

2. Materials and methods

2.1. Sampling area

The sampling sites selected for this study are indicated in Fig. 1:

1. In the northern part an urban-industrial area with an urbanization index of 0.64 (UI being the percentage of the constructed area, Rapoport et al., 1983).
2. An urban-commercial area situated in the center with a UI of 1.0.
3. A residential area situated in the southern part of the city, 20 km from the center, with a low urbanization index (0.15).

2.2. Sampling methods

In 1991, airborne particles $< 10 \mu\text{m}$ (mass concentration) and the proteins associated with these particles were routinely monitored for 1 day a week at three sampling sites in Mexico City. The airborne particles were collected over 24 h on glass fiber filters, $20.3 \times 25 \text{ cm}$, (Gelman Sciences Inc., MI), using three high volume Sierra Andersen samplers (GMW model 1200 VFC HV-PM10) placed on the rooftop of a single story building at a height of about 6 m. According to the manufacturer's specifications, these filters retain particles with a diameter greater than $0.3 \mu\text{m}$ at 95% efficiency. The PM10 samplers were operated simultaneously and the flow rate was kept constant at $1.13 \pm 0.04 \text{ m}^3/\text{min}$ using G313 flow control modules (General Metal Works Inc., OH).

Wind speed values were obtained from the meteorological stations close to the air samplers (Mexican Secretariat of Urban Development and Ecology, SEDUE).

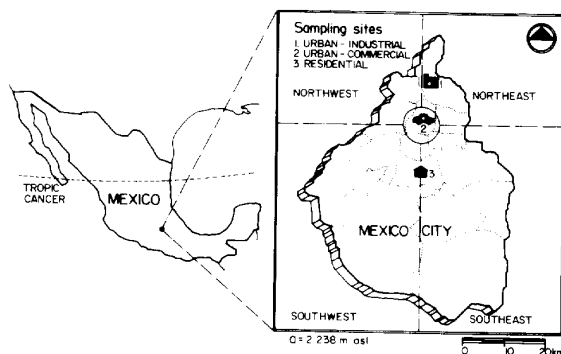


Fig. 1. Location of the sampling sites.

2.3. Air filter analysis

Extraction of proteins. Atmospheric airborne particles concentrations were obtained gravimetrically by weighing the equilibrated filters before and after sample collection; a mechanical milligram balance with a 0.1-mg sensitivity (Sartorius model A 200S) was used.

The air filters were cut into small pieces and the protein moiety was extracted by blending with 120 ml of 0.05 M phosphate buffer + 0.9% NaCl, pH 7.2 at low temperature (4°C) (Twiggs et al., 1982; Preller et al., 1989). Suspended material was removed from the solution using a Buckner funnel, followed by filtering through Millipore $0.45\text{-}\mu\text{m}$ membranes. The final volume of the extract was measured. The protein present in this solution was precipitated by saturating with solid ammonium sulfate, on ice, with constant stirring, and then stored at 4°C overnight. The chilled extract was centrifuged at 19 000 rev./min for 30 min, and the sediment was dialyzed against distilled water. The samples were concentrated by lyophilization to approximately 200 μl . These extracts were used for further analysis. To determine the effect of this treatment on protein extraction, 3.3 and 6.6 mg of plasma proteins, 0.05 and 0.30 mg of bovine serum albumin were applied to four different clean filters and treated in the same way. A recovery of $\sim 60\%$ was obtained. A blank was also prepared using a clean filter sheet, in which case no protein was detected (detection limit $0.03 \mu\text{g}/\text{m}^3$).

Protein determinations. The concentration of protein in samples was determined using Lowry protein assay reagents from Sigma and bovine serum albumin as a standard (Lowry et al., 1951). SDS-PAGE electrophoresis was performed using a PhastSystem (Pharmacia-LKB Biotechnology Inc., Uppsala, Sweden) according to the manufacturer's instructions. Homogeneous (12.5% polyacrylamide) and gradient (8–25% polyacrylamide) gels ($0.45 \times 43 \times 50 \text{ mm}$) were used together with SDS buffer strips. Molecular weights of the bands stained with the silver reagent were determined by means of a LKB 2202 UltroScan Laser Densitometer (Pharmacia-LKB Biotechnology Inc., Uppsala, Sweden).

Isoelectric focusing. Phast Gel IEF 3–9 slabs ($0.35 \times 43 \times 50 \text{ mm}$, pH range 3–9), were run in a Phast-System, according to the manufacturer's instructions (Pharmacia-LKB Biotechnology). Formation of the pH gradient and focusing of the samples took approximately 40 min under constant power (3.5 W). The gels were developed using the Phast Gel Silver Kit from Pharmacia.

2.4. Statistical methods

The raw data with respect to protein concentration, fitted neither a normal nor a log-normal frequency distribution. Therefore, exploratory data analysis techniques were used (Hoaglin et al., 1983). The results from

different sampling sites were compared using box-plot diagrams and non-parametric tests (Kruskall-Wallis). The distribution of the protein values with respect to seasonal influence was represented by stem and leaf diagrams and the rainy and dry season were compared using Wilcoxon's signed-rank test. Spearman rank correlation coefficient was calculated between protein values and airborne particle concentration.

3. Results

One-hundred and fifty samples were collected in Mexico City in order to evaluate the protein concentration associated with airborne particles $< 10 \mu\text{m}$; 66% of the samples contained protein in concentrations $< 0.03 \mu\text{g}/\text{m}^3$ (detection limit, DL). During the sampling time, the wind speed ranged from 0.6 to 6.6 m/s, therefore the collection efficiency of particles $\leq 10 \pm 1 \mu\text{m}$ was not affected (McFarland et al., 1984).

Fig. 2 shows the distribution of the protein levels at different sampling sites, in parallel schematic box-plots. The distributions were asymmetric with a median of 0.18, 0.09 and $0.09 \mu\text{g}/\text{m}^3$ in urban-industrial, urban-commercial and residential zones, respectively, but no significant difference was observed between them (Kruskall-Wallis, $P > 0.05$). However, higher concen-

trations were observed in the urban-industrial zone, with a maximum of $2.5 \mu\text{g}/\text{m}^3$ and only 28% of the filters collected in this site presented protein concentrations lower than the DL.

The seasonal variation of the protein concentration is shown in Fig. 3. In both rainy and dry seasons, the distribution was asymmetric with a mode $\leq 0.03 \mu\text{g}/\text{m}^3$ and a range of ≤ 0.03 –1.2 in the rainy season, while during the dry season, the values ranged from ≤ 0.03 to $2.5 \mu\text{g}/\text{m}^3$. A significant difference was observed between the seasons (Wilcoxon's signed rank-test, $P < 0.05$).

The airborne particles values presented a similar seasonal pattern with higher concentrations in the dry season that ranged from 43 to $247 \mu\text{g}/\text{m}^3$. Fifteen percent of the samples collected in this season exceeded the US airborne particles $\leq 10 \mu\text{m}$ 24-h standard ($150 \mu\text{g}/\text{m}^3$) (Solomon et al., 1989), while only 1% exceeded it during the rainy season (Table 1).

The prevailing northeast wind was recorded and the wind speed ranged from 0.6 to 6.3 m/s in the northern part, from 1.2 to 4.7 in the center and from 1.1 to 5.6 m/s in the southern part of Mexico City. A significant correlation (Spearman rank correlation, $r = 0.50$) between protein concentration and airborne particles was obtained (Fig. 4). This shows that protein concentrations higher than $0.5 \mu\text{g}/\text{m}^3$ were associated with airborne particle values higher than $100 \mu\text{g}/\text{m}^3$, mainly in the urban-industrial site.

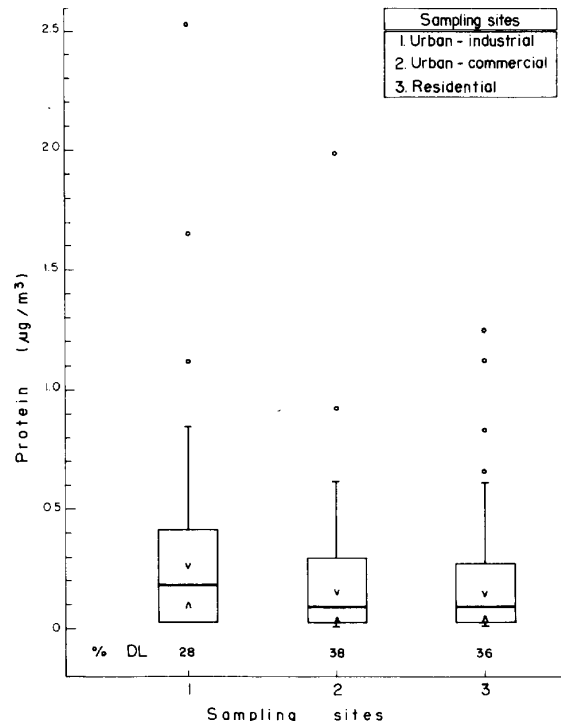


Fig. 2. Schematic box-plot of protein concentration at the sampling sites.

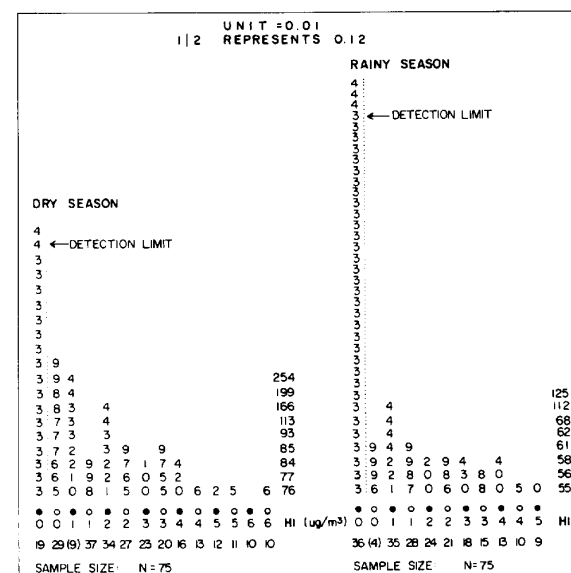


Fig. 3. Stem and leaf diagrams of protein concentrations during rainy and dry seasons. The first row from bottom to top indicates sample size (N), the second row corresponds to accumulated frequency and the third one indicates protein concentrations. The value in brackets is the average.

Table 1
Protein and airborne particle ($\leq 10 \mu\text{m}$) concentrations during the dry and rainy seasons in Mexico City

Variable	Protein ($\mu\text{g}/\text{m}^3$)		Particles ($\mu\text{g}/\text{m}^3$)	
	Rainy ($n = 74$)	Dry ($n = 76$)	Rainy ($n = 74$)	Dry ($n = 76$)
Mode	≤ 0.03	≤ 0.03	76.8	125.1
Geometric mean	0.09	0.15	64.8	120.1
Range	≤ 0.03 –1.2	≤ 0.03 –2.5	28–172	43–247
% < detection limit	7	18	—	—
% Days higher than US airborne particle standard ($150 \mu\text{g}/\text{m}^3$)	—	—	1	15

IEF gel experiments (Fig. 5A) showed that some of the extracts from different sampling sites (two from each site) had proteins with similar isoelectric points ranging from nearly 4.0 to 5.85. This indicates that only acid proteins were extracted from the air filters. On the other hand, the relative molecular weight (Mr) and the heterogeneity of the protein extracts are shown in Fig. 5B. About seven bands were observed in one sample from the urban-commercial area (Mr, 15 000–106 000 Da), six bands in the urban-industrial sample (Mr, 8000–77 000 Da) and three bands in the samples from residential area (Mr, 15 100–30 000 Da). The filter extracts from the urban commercial and residential areas showed one common band with a Mr of $\sim 29\,500$ Da.

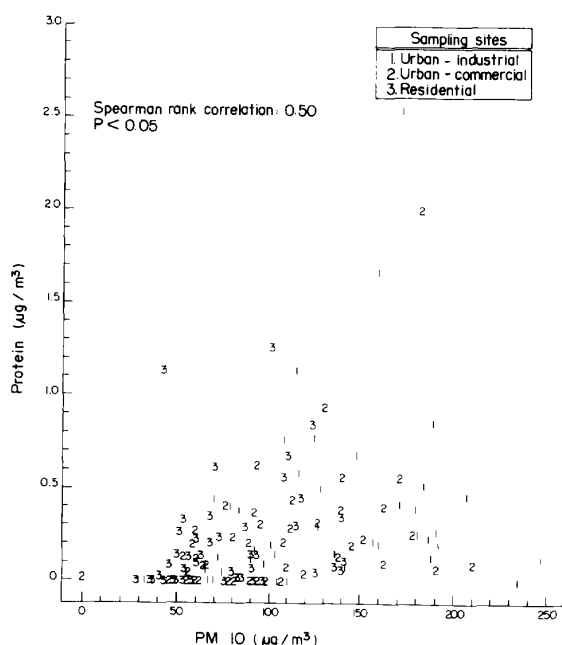


Fig. 4. The relationship between airborne particles and protein concentrations obtained at different sampling sites.

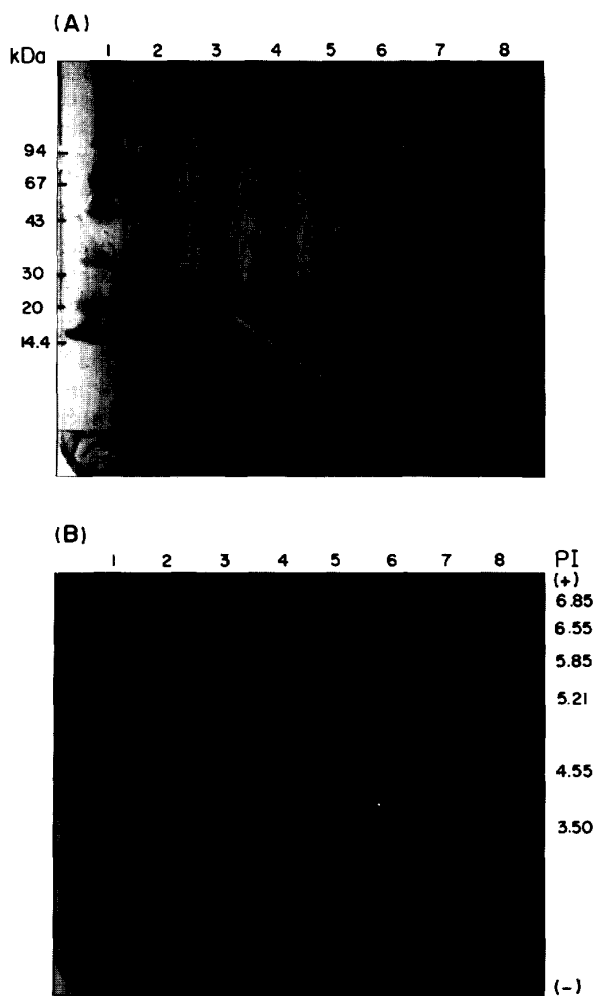


Fig. 5. Electrophoretic experiments on the PhastSystem. (A) SDS PAGE. Lanes 1 and 5, Low Molecular Weight Calibration Kit (94, 67, 43, 20, 21, 14.4 kDa). Lanes 2 and 3, protein extracts from filters of the residential area (0.5 and 0.21 μg , respectively). Lanes 4 and 6, protein extracts from the urban-commercial area (0.36 and 0.15 μg , respectively). Lanes 7 and 8, extracts from the urban-industrial area (0.1 and 0.6 μg , respectively). (B) IEF. Lanes 2 and 3, protein extracts from filter of the residential area (0.36 and 0.25 μg , respectively). Lane 5, Broad pI Calibration Kit (pI: 3.5, 4.55, 5.2, 5.85, 6.55, 6.85, 7.55, 8.15, 8.45, 8.65 and 9.3). Lanes 7 and 8, extracts from the urban-industrial area (0.5 and 0.6 μg , respectively).

4. Discussion

Proteins are one of the most abundant and diverse classes of antigens to which the immune system can respond, and this is related to their permeability and immunogenicity. The evaluation of protein associated with airborne particles has been used in both indoor and outdoor environments as a method of indicating an increased concentration of potential allergens (Agarwal et al., 1981; Twigg et al., 1982; Preller et al., 1989).

In this study, we analyzed air filters from three areas with different urbanization indices. Concerning protein levels, no significant differences were observed between the three areas. Surprisingly, the highest geometric mean ($0.15 \mu\text{g}/\text{m}^3$) was recorded in the urban-industrial area. This result was expected for the residential area, where the largest green area of the city is present and higher concentrations of pollen and spores have been reported (Calderón et al., 1993; González-Macias et al., 1993). In general, the geometric mean value obtained during the rainy season ($0.09 \mu\text{g}/\text{m}^3$) in Mexico was similar to that reported upwind ($0.11 \mu\text{g}/\text{m}^3$) of a yeast and penicillin production plant in the Netherlands (Preller et al., 1989). Moreover, during the dry season the protein values increased like the ones downwind from the same industrial plant. Therefore, it is possible that any increase in the airborne protein concentration could be a good indication of biological materials, whose specific source is unknown. On the other hand, it was very difficult to relate the protein concentrations, molecular weight, and isoelectric points, to those associated with specific immunological disorders. However, in Mexico City the inhalation of biological material and a great variety of toxic substances must be investigated in order to evaluate the effects of air pollution on the population's health.

A positive and significant correlation ($r = 0.50$) between protein concentration and airborne particles was obtained. Values up to $240 \mu\text{g}/\text{m}^3$ of airborne particles were recorded both in urban-industrial and urban-commercial areas, where many unpaved roads, reduced green areas, heavy traffic, as well as high levels of air pollution are common. Besides, the soil in these areas is being continuously polluted with human and animal excreta. This suggests that the resuspension of road dust could be an important source of airborne proteins whose allergenic properties have been investigated (Taylor et al., 1977; Schumacher, 1980).

The seasonal distribution analysis of airborne particles and protein concentrations showed a significant difference between rainy and dry seasons, with higher concentrations during the dry season. This might be the result of the low moisture of the soil that increases the potential aerosolization of the particles.

Molecular weights of the proteins extracted from the air filters ranged from 8000 to 106 000 Da, but the aver-

age did not exceed 65 000. The last value represents the upper limit of permeability of the mucosal membrane (King, 1976). A protein of 29.5 kDa was common in filters from urban-commercial and residential areas, but it was not present in the filters from the urban-industrial area. The isoelectric points of these proteins were in the range of 4–5.85 indicating that all were acidic. Protein with these characteristics has been detected in several allergen sources, as perennial rye grass (*Lolium perenne*) pollen (Ansari et al., 1989). The combination of low molecular weight and acid isoelectric point may include these proteins from outdoor environments among the major aeroallergens, but whose specific source is unknown (Benjamin et al., 1984).

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