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PIXE Analysis Of Atmospheric Aerosols Related To Their Cytotoxic And Genotoxic Effects

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ABSTRACT

Results are given of a study regarding the relationship of composition of airborne particles in Mexico City (PM₁₅, PM₁₀ and PM_{2.5}) with citotoxic/genotoxic effects on cell cultures. Elemental analysis of samples collected during the first half of 2002 at three sites was carried out with PIXE, together with several citotoxic/genotoxic studies (generation of •OH, cellular death, inflammatory responses, and damage to cellular DNA). Differences were found in elemental contents in the aerosols from the three sites. The results suggest that some of the latter effects are correlated to higher concentrations of certain elements in the particles.

Keywords: Aerosols, Mexico City, Cytotoxicity, genotoxicity.

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1. INTRODUCTION

The air pollution problem in the Metropolitan Area of Mexico City (MAMC) has been extensively studied [1]. In particular, particulate matter (PM) has been related to effects in human health, which include decrease in pulmonary capacity, asthma, and increase in mortality [2]. However, the mechanisms relating the presence of the particles and the disease development are not well understood. There is experimental evidence regarding damage to cell cultures and laboratory animals [3]. With this in mind, a wide study aimed to obtain detailed information about the composition (anthropogenic chemistry, soil and biological contaminants) of airborne pollutants in Mexico City and their potential for inducing cell damage was conducted recently [4]. In the present work, only results regarding elemental contents in the airborne particles, as measured with PIXE and their relationship with citotoxic and genotoxic effects of the particles on cell cultures are given.

2. MATERIALS AND METHODS

Samples of atmospheric aerosols were collected in three sites in the MAMC. Particles for PIXE analysis were deposited onto polycarbonate filters (Nuclepore, Costar Corp.) using Stacked Filter Units (SFU) of the Davis design [5], every second day, along 24 h, (8:00 h to 8:00 h of the next day), from January 18th to August 1st, 2002. Particles for citotoxic/genotoxic studies were simultaneously collected with High-Vol samplers (Sierra-Andersen), deposited onto nitrocellulose filters. SFU collected PM₁₅, while the High-Vol samplers worked for PM₁₀ and PM_{2.5} collection. The devices were always located at roof top level (between 2.5 m and 15 m). The sampling sites were placed in the North (FES-Iztacala, UNAM), Center (the RAMA station in La Merced), and South (Centro de Ciencias de la Atmósfera, main UNAM campus). SFU fine and coarse fractions were analyzed with PIXE, using a 2.2 MeV proton beam produced by the Pelletron accelerator at IFUNAM. The protocols, including the irradiation chamber and detection system efficiency measurement with MicroMatter (Deer Harbor, WA, USA) thin film standards, are described elsewhere [6, 7]. To verify analytical accuracy, a second set of thin film standards was irradiated, resulting in a maximum error of 0.8 %. Also, gravimetric mass was determined using a 210D Ohaus electrobalance (resolution 10 µg), as reference. The citotoxic/genotoxic effects considered in this work were generation of •OH, cellular death, inflammatory responses, and damage to cellular DNA. Procedures for the study of these effects are detailed in a different paper [3].

3. RESULTS AND DISCUSSION

Mean elemental concentrations measured with PIXE are summarized in Figure 1 for the three sites, fine and coarse fractions, respectively. Also, analysis of variance was applied to determine whether the elemental concentrations in each site were significantly higher or lower. It was found that in the fine fraction Zn and Se, anthropogenic elements, are significantly lower in the South, while K and Cu are higher in the Center site. K may be associated to a contribution from the Texcoco Lake fugitive dusts and the emission of smoke from street vendors, something already observed in a previous work [8]. The source for Cu must be further investigated. Regarding the South site, only S, Fe, and Pb are significantly lower. With respect to the coarse fraction, more elements are significantly higher in Center (Si, Cl, Ca, Mn, Fe, and Cu); while the first five elements may be associated to soil dust, the latter is consistent with the results in the fine fraction. It means there is an important source of Cu in the Center. Additionally, the South site contains many significantly lower concentrations of all elements, except Ni and Cu. This is in agreement with other findings, where South had lower contents of aerosols than Center or North [8]; Pb was not observed in this site.

The multivariate statistical analysis technique Absolute Principal Component Analysis (APCA) was also applied to all the data sets, as explained in a previous work [9]. Table I presents the sources that contribute to each fraction and site, together with the elements associated to the sources.

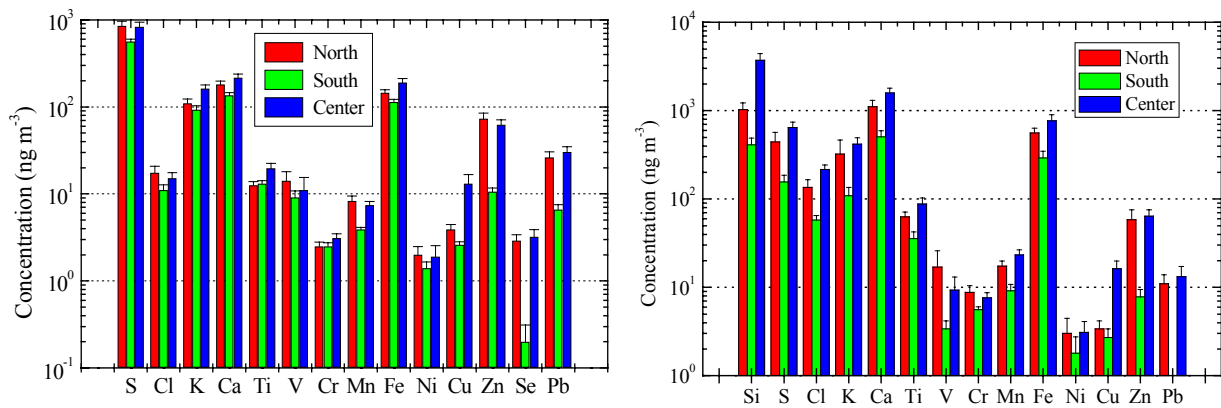


FIGURE 1. Mean elemental concentrations measured for the (a) fine and (b) coarse fractions.

TABLE 1. SOURCES IDENTIFIED WITH APCA IN EACH SITE AND ELEMENTS PRESENT IN THEM.

Site	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
North, Fine Fraction	Soil S, K, Ca, Ti, Fe	Industry metal Cl-Cu	Fuel S, V, Ni	Industry 1 Cr, Se, Pb	Industry 2 Mn, Fe, Zn, Se
North, Coarse Fraction	Fuel S, Cl, Ca, V, Cr, Ni	Soil Si, Ca, Ti, Mn, Fe	Smoke S, K	Industry Cu, Zn, Pb	
Center, Fine Fraction	Fuel S, V, Ni	Soil Ca, Ti, Mn, Fe	Industry Cu, Zn, Pb	Salt Cl, K	
Center, Coarse Fraction	Soil Si, S, K, Ca, Ti, Mn, Fe	Fuel S, V, Ni	Sulfate S, Cl	Industry Cu, Zn, Pb	
South, Fine Fraction	Soil S, Cl, K, Ca, Ti, Mn, Fe	Industry 1 Cu, Zn, Se, Pb	Fuel S, V, Ni	Industry 2 Cr	
South, Coarse Fraction	Soil Si, S, Cl, K, Ca, Ti, Mn, Fe	Fuel S, V, Ni, Cu, Zn	Industry V, Cr		

It must be noted that S appears as a component of Soil, because there are important sources of this element (possibly as SO₂) in the MAMC (for example, the Popocatepetl Volcano and fuel). The presence of S "attached" to soil particles was corroborated with electron probe microanalysis [4].

Relative contributions from sources identified with APCA indicate that Soil is the main contributor to the measured mass, in agreement with previous studies [8, 9]. Moreover, contributions from Fuel must not be overlooked, as regulatory measures banned the use of fuels containing V and Ni; the presence of these elements correlated to S, show that these materials are still consumed in the MAMC.

As endotoxins were found in airborne particles, it was necessary to assess their role in toxic processes. The activation of the complement system (molecules that participate in the defense of an organism) is a known effect of endotoxins in humans. In this case it was observed that the particles activated the complement system with no significant differences by sample site or concentration of the endotoxins. Therefore, there must be more components in PM capable of activating the complement system, besides endotoxins

The potential for PM₁₀ to cause damage by oxidation may be seen through the generation of $\cdot\text{OH}$. This was measured by electron paramagnetic resonance using DMPO and required the presence of H₂O₂, suggesting the participation of Fenton type reactions. The generation of $\cdot\text{OH}$ abolished with deferoximene proved the role of metals. This result correlates with the metal concentrations in samples from the three sites (C > N > S).

The induction of cellular death also correlates with a greater concentration of metals in the particles. Those from the Center were the most cytotoxic. Regrettably, the application of deferoximene in the cell system caused an indefinite inhibition of the cellular function, not allowing a stronger conclusion regarding the effect of metals in cell damage.

The induction of inflammatory responses mediated by cytokines may be another of the processes by which PM are probably harmful. TNF α and IL-6 were measured as indicators of the participation of proinflammatory cytokines. The production of cytokines was induced by all samples according to the elemental concentrations found in the particles. Those from the Center site were the most potent. An inhibitor of endotoxins blocked its effect in part, suggesting the contribution of different PM components in the process.

Regarding a different mediator of inflammation, prostaglandin E₂ (PGE₂), the behavior was different. This time, the particles from the South were more effective and the inhibitor of endotoxin entirely blocked the outcome.

A further aspect in the study showed the capacity that PM has to induce damage to genetic material in cells (genotoxicity). The generation of DNA breaks in cells exposed to particles was measured by the comet technique [4], and it was found that particles from the northern and central sectors were potent inducers of DNA breaks. Nevertheless, data about the production of p53 (the molecule involved in the detection and repair of those damages), indicate that the mechanisms for destruction are different. As only particles from the South induced the production of p53, it is believed that the induced damage by the particles from the North and central regions are so strong that there is not enough time to trigger a repair response. It may be linked to the concentration of metals and the creation of $\cdot\text{OH}$.

Emphasis must be given to the error introduced when comparing results obtained from different devices and methods designed for collection of particles in varying size ranges. The collection efficiency curves are somewhat different. SFU samplers tend to underestimate the mass as compared to another "reference" method employed in the study and sanctioned by the US Environmental Protection Agency (EPA) [4]. The device used to study biological and toxicological effects has a behavior similar to the reference method.

4. CONCLUSIONS

Significant differences in the elemental concentrations were found for the three studied sites. Cytotoxic, inflammatory and genotoxic effects induced by PM were also observed. The relative intensity of these effects seems to correlate with the presence of metals (for example, cellular death). However, in other cases the results are not easily understood in terms of elemental contents. Further studies must be conducted to explain them, including the use of other sampling devices to have a better agreement in the size and mass of the collected particles.

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