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Characterization and in vitro biological effects of concentrated particulate matter from Mexico City

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Abstract

Coarse and fine particles were collected using an ambient particle concentrator (VACES system) in the north, center and south regions of Mexico City during May and November of 2003 with the aim of collecting enough particulate matter (PM) to examine their chemical and physical characteristics, biological content, and toxicity potential. The chemical, morphological and biological composition of PM was determined, together with the redox activity, induction of apoptosis and DNA damage. Carbonaceous species determined by thermal-optical transmittance (TOT) showed that the highest concentrations were found in $PM_{2.5}$ from the north and in PM_{10} from the center. When analyzed by inductively coupling plasma (ICP), levels of metals were higher in the coarse fraction, mainly in the north. Morphological analysis by Scanning Electron Microscope & Energy Dispersive X-ray Spectrometer (SEM-EDX) is shown. Bacteria, fungi and endotoxin were present mostly in the coarse samples from the north. Fine PM had higher redox activity, than the coarse PM assessed by the dithiothreitol (DTT) assay. Early apoptotic cell death assessed by annexin V was observed in A549 cells exposed to PM from all regions, particularly with those collected in May. The fine fraction from the south induced higher apoptotic cell death compared to the coarse fraction, in contrast, the coarse fraction from the north induced significantly higher apoptosis than the fine fraction. All PM samples induced DNA damage assessed by the comet assay on THP-1 cells when exposed to a concentration of 10 μg/mL, the highest DNA damage was produced by both particle fractions collected in the

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north in May and November. In conclusion, PM from the north showed a higher metal and biological content, apoptotic cell death induction and more extensive DNA damage. Also, fine PM fractions from all sampled regions showed more redox activity than the coarse fraction. In summary, location, season and size of PM collection influenced their chemical, morphological and biological composition and thus their toxicity to cells.

Keywords: Ambient particulate; Chemical composition; Dithiothreitol assay; Apoptosis; DNA damage; CAPs and in vitro

1. Introduction

Exposure to ambient particulate matter (PM) represents a significant health risk in major urban centers, such as Mexico City (Téllez-Rojo et al., 2000; Pope et al., 2002). The chemical and physical properties and toxicological mechanisms by which PM causes adverse health effects are still uncertain. PM aerodynamic size has become a relevant factor when studying PM toxicity due to its ability to penetrate the respiratory system; i.e., fine particles reach the deeper regions of the lungs, whereas coarse PM may be deposited in upper regions of the airways. PM chemical components mediate toxic response and could thus be relevant in the induction of adverse health effects in humans (Ghio, 2004). Previous work showing the high content of metals in coarse and fine PM from Mexico City (Mugica et al., 2002) suggests they could play an important role in damage to cells. Fine PM potentially may owe its toxicity to its content of organic compounds, metals and other reactive chemical compounds (Lighty et al., 2000). Alfaro-Moreno et al. (2002) reported that *in vitro* studies in human pulmonary cells and murine macrophages exposed to PM_{10} from the northern and central regions of Mexico City showed higher toxic effects than those from the southern region (cytotoxicity, DNA breakage and apoptotic cell death).

A complex challenge to study the underlying factors in PM toxicity is to obtain enough PM to perform controlled laboratory studies. To address this issue the southern california particle center and supersite (SCPCS) has developed a versatile aerosol concentration enrichment system (VACES), allowing collection of large amounts of PM by size fraction. The size fractionated PM is collected in concentrated PM suspensions by connecting the output flows of each parallel concentrator to a liquid impinger (BioSampler^{TM}), used in a modified configuration in order to collect particles under near-ambient pressure (Kim et al., 2001). The SCPCS joined efforts with air pollution researchers from four Mexican institutions: Centro de Investigación y de Estudios Avanzados (CINVESTAV), Universidad Autónoma Metropolitana (UAM-A), Universidad Nacional Autónoma de México (UNAM) and Instituto Nacional de Cancerología (INCan), to install and operate for the first time the VACES system in Mexico City, integrating an interdisciplinary group of scientists specialized in PM studies (Mexican Consortium for particulate matter studies).

The toxicological evaluation of PM exposure is often limited by the amount of PM that can be collected, therefore the aim of this study was to collect PM from ambient air in Mexico City with a particle/virtual impactor concentrator to examine them for their chemical and physical characteristics, biological content, and potential toxicity. PM were collected in the northern, central and southern regions of Mexico City in May and November of 2003 and their carbon and metal content, morphology and redox activity were determined, together with their cytotoxic potential assessed by apoptotic cell death and DNA damage induction.

2. Material and methods

2.1. PM collection

Coarse (2.5–10 μm) and fine (<2.5 μm) PM were collected in the north—industrial (Xalostoc), center—commercial (Merced) and south—residential (Pedregal) regions of Mexico City during 4 continuous days in May and November of 2003, representing the warm and cold-dry seasons. Particles were collected using the particle concentrator VACES system, which consists of three parallel sampling lines, each one operating with an intake flow rate of 110 L/min (Kim et al., 2001). Briefly, coarse particles were collected by concentration using a single nozzle virtual impactor and fine particles were concentrated by drawing air samples through two parallel lines using 2.5 μm cut-point preimpactors to remove larger size particles. Enriched particle suspensions are obtained in

aqueous solutions by connecting the VACES output to ultrapure water as a collection medium (BioSampler, SKC West, Inc., Fullerton, CA) (Li et al., 2003). Samples from the 4 days were pooled before analysis and toxicological evaluation.

Parallel PM_{10} and $PM_{2.5}$ samples were collected on Teflon filters using MiniVol samplers (Airmetrics, Eugene, OR) at a flow rate of 5 L/min for the 8 h sampling period to estimate ambient PM levels.

2.2. Mass determination

In order to express the metal, endotoxin and toxicological results on a microgram basis mass was determined from particles collected with the VACES system as follows, after solution sonication 0.5 mL aliquots of the concentrated pooled samples were placed in sterile aluminum cuvettes to let suspension evaporate during 4–5 days under 45% humidity and $20\pm2\,^{\circ}C$ constant temperature conditions. The aluminum cuvettes were weighted pre- and post evaporation in a microbalance (MT/UMT, Mettler) (0.001 mg).

2.3. Particle chemical composition and morphological analysis

PM_{10} and $PM_{2.5}$ samples were collected on quartz filters using MiniVol samplers as previously described for the 8 h sampling period to quantify carbon content. Elemental and organic carbon was determined by thermal-optical transmittance (TOT) with a carbon analyzer (Sunset Laboratory) using the NIOSH protocol.

Metal content was directly measured from the coarse and fine PM VACES solution by acid-extraction of metals using an OI-Analytical microwave oven to quantify ten trace metals by inductively coupling plasma (ICP-AES, Atom Advantage Thermo Jarrel Ash).

For morphological analyses particles obtained with the VACES system were filtered (nylon membrane—0.45 μm pore) and the particles from the filtered solution were adhered over conductive carbon tape with a JEOL5900LV scanning electron microscope equipped with energy dispersive X-ray microanalysis system.

2.4. Biological and endotoxin content

2.4.1. Microbiological analysis

Airborne PM collected with the VACES system and blank water samples were analyzed for cultivatable bacteria and fungi. Three 1:10 serial dilutions were performed. 100 μL aliquots were plated on trypticase soy agar for cultivatable total bacteria, McConkey agar for gram-negative bacteria, and 300 μL aliquots on malt extract agar for total fungi.

2.4.2. Endotoxin measurements

Airborne PM collected with the VACES system and blank water samples were analyzed for endotoxin content, 1 mL aliquots were sonicated for 1 h and vigorously vortexed for 1 min every 15 min. Suspensions were diluted 1:5 with 5 mM TRIS buffer and endotoxin was determined using the Kinetic Quantitative Chromogenic Limulus Amebocyte lysate kit (Kinetic-QLC, BioWhittaker, Inc). Absorbance was read at 405 nm every 150 s in an automated Kinetic-QLC reader at 37 °C, with a detection limit of $0.05\,EU\,mL^{-1}$, following the manufacturer's specifications. *Escherichia coli* O55:B5 endotoxin was used as a standard ($9\,EU\,ng^{-1}$) (BioWhittaker, lot no. 2L1850).

2.5. Oxidative activity

The redox activity of compounds contained in PM samples was assessed by its ability to catalyze the reduction of oxygen by dithiothreitol (DTT) as described in Li et al. (2003) and Cho et al. (2005). The remaining thiol was allowed to react with DTNB, generating 5-mercapto-2-nitrobenzoic acid which is measured by its absorption at 412 nm of optical density. Briefly, coarse ($40\,\mu g\,mL^{-1}$) and fine ($10\,\mu g\,mL^{-1}$) PM samples were incubated at 37 °C with 0.5 M PBS, pH 7.4, double deionized water and 1 mM DTT for 0–45 min. An aliquot of the incubation mixture was mixed with 10% trichloroacetic acid to stop the reaction, and a portion of the mixture was dissolved with a Tris buffer at pH 8.9, 20 mM EDTA and 10 mM DTNB solution. The redox activity is expressed as the rate of DTT consumption (nmol) per minute per microgram of sample minus the activity observed in the absence of PM.

2.6. Apoptosis detection by annexin V-FITC kit

Human lung epithelium A549 cells (ATCC) were used to assess apoptosis by the phosphatidylserine staining detection method of Annexin V-FITC (BD Pharmigen, Biosciences) as described in Alfaro-Moreno et al. (2002). Live cells are represented as annexin V−/PI−, early apoptosis as annexin

V+/PI−, late apoptosis as annexin V+/PI+ and necrosis annexin V−/PI+. Cells were grown as a monolayer and maintained under standard conditions in 10% FBS-Dulbecco's modified Eagle medium (DMEM) (Sigma)+antibiotics. A549 cells were seeded at $2.1 \times 10^5\,cm^2$ and exposed for 24 h to $80\,\mu g\,cm^2$ of concentrated PM collected in an aqueous solution with the VACES system. This concentration was based on previous data from A549 cells exposed to PM from Mexico City (Alfaro-Moreno et al., 2002). Annexin V assay was performed by flow cytometry (FACSort, Beckton Dickinson, San Jose, CA) and the data analyzed with WinMDI V2.8 software.

2.7. Comet assay for DNA damage

Human monocytic cells THP-1 (ATCC) were used to evaluate DNA damage by agarose gel cellular electrophoresis (Singh et al., 1988). Suspensions of 6.5×10^5 cells were exposed under gentle agitation to $10\,\mu g\,mL^{-1}$ (2 mL final volume) of concentrated PM for 24 h. Twenty seven thousand cells were mixed in 1% low fusion point agarose and placed on a covered slide with solidified agarose as described in Alfaro-Moreno et al. (2002).

2.7.1. Statistical analysis

DTT assay and apoptotic cell death analysis were performed in triplicate and in two independent experiments, and data analyzed by the Mann–Whitney test using STATA 8.0. For DNA damage analysis data are expressed as median, maximal, minimal, 25th and 75th percentile of triplicate independent experiments and analyzed by a one way ANOVA using STATA 6.0. Differences were considered significant when $p<0.05$.

3. Results and discussion

3.1. Particulate carbon and metal content

Ambient PM_{10} concentrations during the sampling period were 115.64, 110.84 and 55.91 $\mu g\,m^{-3}$ in the north, center and south, respectively. $PM_{2.5}$ levels were 47.22, 70.8 and 33.4 $\mu g\,m^{-3}$, respectively. The total amount of coarse and fine PM from the 4 days of the particle concentrator collection ranged from 20 to 59 mg and from 14 to 38 mg, respectively. Total carbon (TC), the sum of organic (OC) and elemental carbon (EC), accounted for 20–39% of PM_{10} mass, and for 27–58% of $PM_{2.5}$ mass. The highest concentrations of both carbon species were found in $PM_{2.5}$ from the north, and PM_{10} from the center (Fig. 1). Carbonaceous species showed no clear seasonal variation. The OC/TC ratios were similar for both particle sizes and higher in November. For PM_{10}, the average ratios were 0.81 and 0.84 in May and November, respectively, whereas the $PM_{2.5}$ OC/TC ratios were 0.8 and 0.85 in May and November, respectively. These ratios were higher than those determined during spring 1997 by Chow et al. (2002), who reported 0.69 and 0.63 ratios for PM_{10} and $PM_{2.5}$, respectively.

The measured trace metals accounted for 16% and 13% of the total mass for coarse and fine fractions, respectively. Fe, Zn and Ti were the most abundant metals determined in concentrated PM

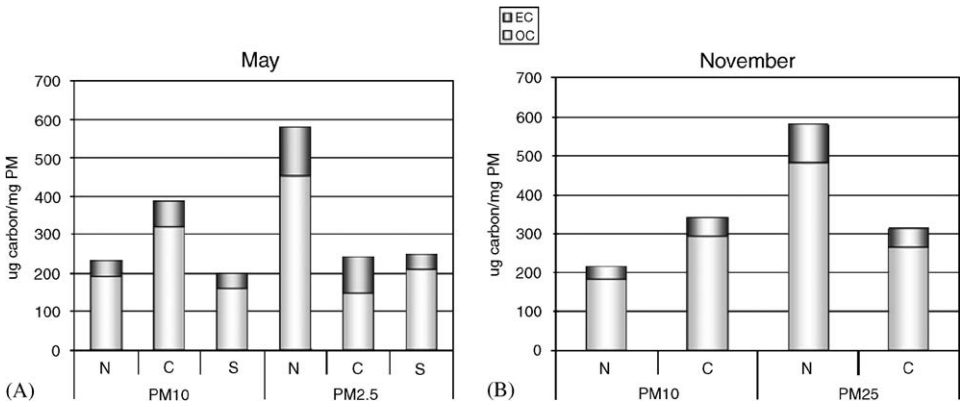

Fig. 1. Content of carbonaceous species determined by TOT carbon analyzer using the NIOSH protocol in PM from Mexico City collected in filters during (A) May and (B) November of 2003.

(Fig. 2) representing around 98% of total metal content, followed by smaller amounts of Mn, Cu, V, Cd, Ni, Cr and Pb. Spatial variation of trace metals is described in Fig. 2. The highest metal concentrations were found in PM collected in the north with concentrations being two fold higher than in the central region. In all cases, the metal content was 30% higher in November. These results are in agreement with studies reporting the northern region, located in a highly industrial area, as the most polluted by PM, with higher concentrations in autumn and winter (Chow et al., 2002; Mugica et al., 2002).

3.2. Morphological analysis

Fig. 3 shows features of PM collected with the VACES system. Image 3A and 3B show coarse and fine particles, respectively, obtained in the center of Mexico City. Image 3D, portrays the crustal chemical composition obtained with EDX spectra of Image 3B. Finally, Image 3C exhibits a rounded particle from the fine fraction of the north, where the EDX analysis reported that iron and oxygen predominate in the overall composition (data not shown) with some carbon content suggesting that the particle could originate from a combustion process.

3.3. Bacteria, fungi and endotoxin content in PM

Table 1 shows the presence of airborne bacteria and fungi in concentrated PM collected during November of 2003 in Mexico City. A higher presence of cultivatable bacteria and fungi, in average 10.8- and 8.27-fold, respectively, was observed in the coarse fraction as compared with the fine fraction. Regional differences in PM biological composition between regions were observed; PM collected in the north had 1.8 and 1.5 times more cultivatable bacteria and fungi, respectively, than those collected in the central region. There was no significant presence of gram-negative bacteria in all the samples collected.

Endotoxin content in concentrated PM from Mexico City is shown in Table 2. All samples showed detectable levels of endotoxin. Endotoxin levels in the coarse fraction were between five to 50 times higher than in the fine fraction in PM collected in the center and the south of the city; however, only a two-fold difference was observed in the north. PM collected in May from the central region had the highest endotoxin levels 895 EU mg^{-1}, followed by those from the north (655 EU mg^{-1}) and south (165 EU mg^{-1}). The levels present in the coarse fraction of PM collected in November were lower than those collected in May,

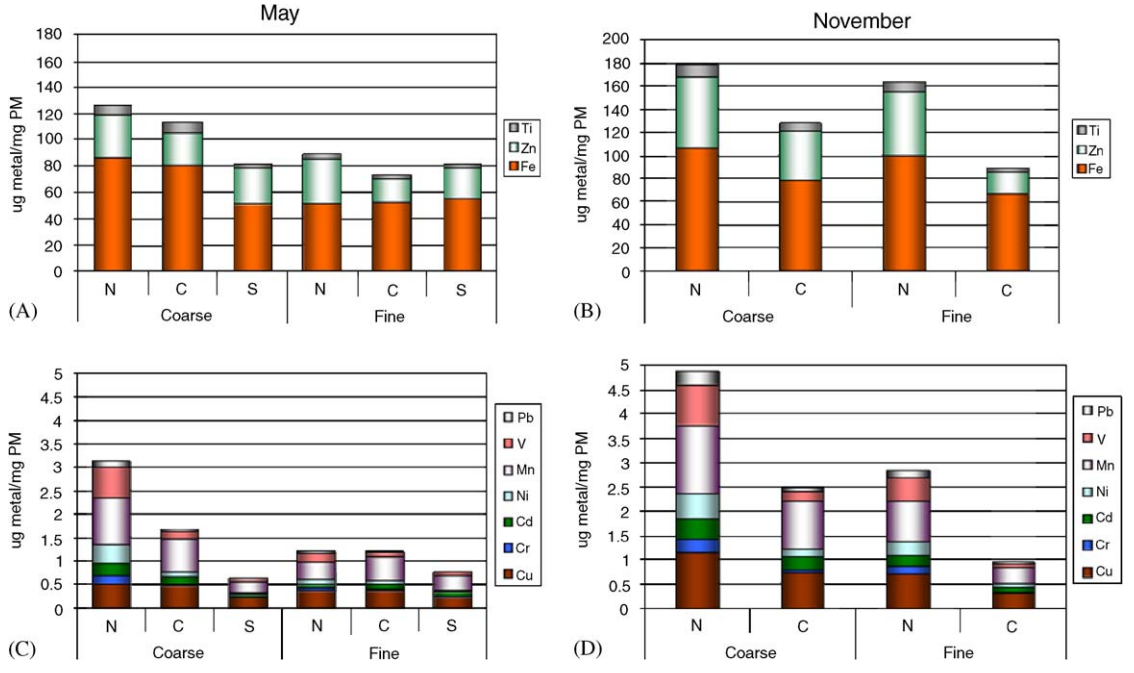

Fig. 2. Metal content of concentrated PM and determined by ICP-AES during (A and C) May and (B and D) November.

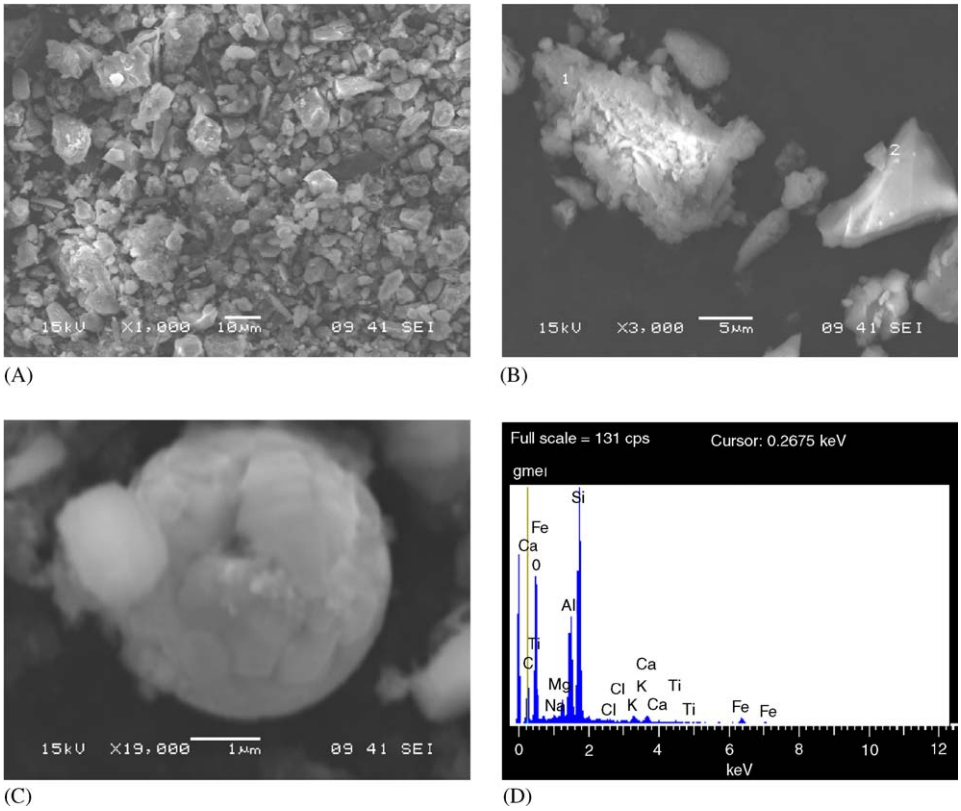

Fig. 3. (A) Coarse and (B) fine crustal concentrated particles. (C) FeO particle from combustion process collected with VACES. (D) Composition spectra of B.

Table 1
Bacteria and fungi content in PM collected in Mexico City during the November season of 2003

VACES (CFU mg^{-1})	Water blank (CFU mL^{-1})	North		Center	
		Coarse	Fine	Coarse	Fine
Trypticase soy agar	10	10517	1154	5620	449
McConkey agar	0	0	0	0	0
Malt extract agar	140	25172	1758	20496	9132

Fungi: *Alternaria* sp., *Cladosporium* sp., *Aspergillus* sp., *Rhizopus* sp., *Penicillum* sp., *Monilia* sp.
Bacteria: *Actinobacterium* sp., *Staphylococus* sp., *Bacillus* sp.

813 EU mg^{-1} in the center and 606 EU mg^{-1} in the north. These data are in agreement with previous reports indicating that endotoxin is present in PM_{10} collected in filters in Mexico City (Osornio-Vargas et al., 2003). The biological content identified in the coarse fraction of PM from Mexico City could participate in pro-inflammatory processes involved in PM *in vivo* exposures as reported by Schins et al. (2004). In the endotoxin analysis less than

Table 2
Endotoxin content in PM collected in Mexico City during May and November of 2003

VACES (EU mg^{-1})	North		Center		South	
	Coarse	Fine	Coarse	Fine	Coarse	Fine
May	655	387	895	50	165	30
November	606	25	813	16	—	—

1 EU mL^{-1} was determined in the water used as a negative control and 84.2 EU mL^{-1} in the water obtained from the VACES saturator.

3.4. Redox activity of PM

On a microgram basis, a two-fold higher redox activity of the fine fraction from all regions compared to the coarse fraction was observed (Fig. 4), in addition, fine PM collected in the center in May and in the north in November were the most redox active. Our data shows that the redox activity follows a similar behavior as the carbon content levels in PM collected in the center and north in November. However, the same was not observed for PM collected in May where only PM from the north showed a similar behavior as the carbon content. The redox activity identified in PM has been related to their capacity to induce oxidative stress in cell systems, as reported by Li et al. (2002) for PM obtained in Los Angeles basin. Thus, on a microgram basis, the redox activity identified in this study in the fine fraction of ambient PM collected in Mexico City could potentially contribute to oxidative cell damage.

3.5. Apoptotic induction in epithelial cells exposed to PM

Particles collected in May and November in the north, center and south of Mexico City induced between 5.0% and 20.6% of early apoptotic cell death (annexin V+/PI−) in A549 cells. Representative flow cytometry histograms of control and cells exposed to the fine fraction of PM from the north are shown in Fig. 5(A). Early apoptosis (annexin V+/PI−) and late apoptosis (annexin V+/PI+) events are illustrated in Fig. 5(B), where the fine fraction of PM collected during May in the south showed a small but significant increase in apoptotic cell death than the coarse fraction. In contrast, the coarse fraction from the northern region induced significantly higher apoptotic cell death than the fine fraction when compared per microgram. In this respect, other studies have also observed an equal cytotoxic (measuring cytokines and apoptosis) contribution by mass of the larger size fraction of ambient air particles with respect of the smaller fraction (Hetland et al., 2004). No significant differences were observed between the fractions collected in the center. Exposure to coarse PM collected in November did not show significant differences between the north and the center ($\approx$10%). Our results showed that fine PM collected in the center and the south of Mexico City in May were able to induce significant apoptotic cell death, however, both fractions of PM collected in the north induced apoptosis. Our overall results are in agreement with previous reports of apoptotic cytotoxicity in A549 cells observed by particles (PM_{10}) from the north collected in filters (Alfaro-Moreno et al., 2002).

3.6. DNA damage in human monocytic cells

Coarse and fine concentrated PM from Mexico City from all regions induced DNA damage as assessed by the comet assay in THP-1 cells (Fig. 6). Maximal comet length was induced by both PM fractions collected in the north, particularly by the coarse fraction from the north where more than a 75% of DNA damage ($p<0.002$) was observed. Exposure to coarse PM collected in May showed no significant differences between regions, whereas fine

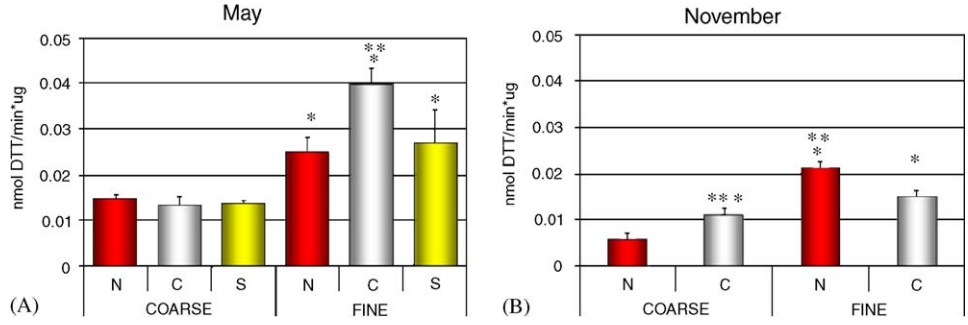

Fig. 4. Redox activity of concentrated PM assessed by the dithiothreitol (DTT) assay. $N = 6 \pm$SD, *$p<0.05$ fine vs. coarse, **$p<0.05$ north fine vs. center fine, ***$p<0.05$ north coarse vs. center coarse. Activity is expressed as the rate of DTT consumption (nmol) $min^{-1}\,\mu g^{-1}$ of sample minus the activity observed in the absence of PM.

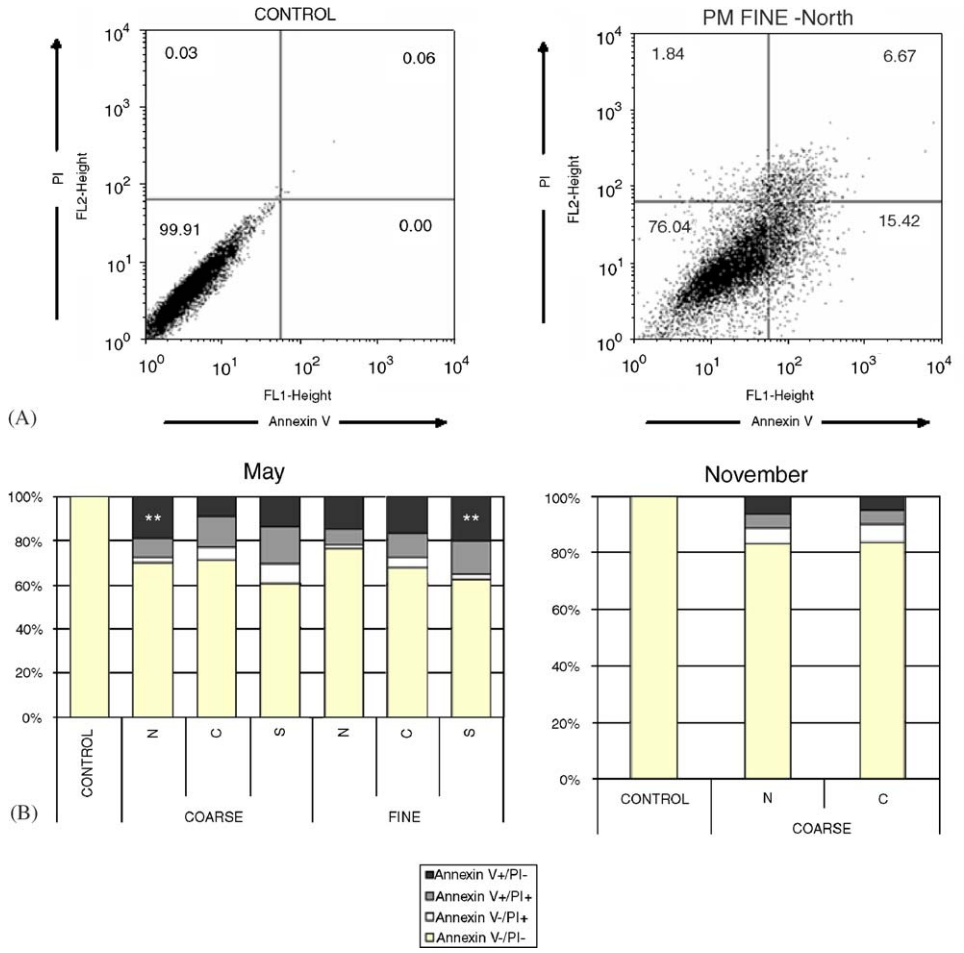

Fig. 5. Flow cytometry histograms of A549 cells showing: (A) control and exposed to 80 μg cm^{-2} of PM from Mexico City. (B) Data expressed in percentage of apoptosis induced, differences between PM size and regions can be observed. Values are a mean of two independent experiments in triplicate. Al values presented $p<0.05$ when compared to controls; also $^{**}p<0.05$ fine vs. coarse.

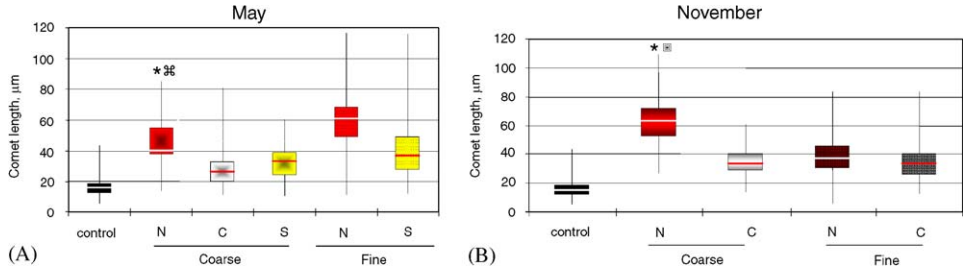

Fig. 6. Distribution of comet length induced in THP-1 cells exposed 24 h to 10 μg mL^{-1} of concentrated coarse and fine PM collected in Mexico City. Data are expressed as median, maximal, minimal, percentile 25 and 75 ± SD, of triplicate independent experiments and analyzed by a one way ANOVA. Differences were considered significant when $p<0.05$, $^{*}p<0.002$, $^{⌘}p<0.007$, $^{▣}p<0.0001$.

PM collected in the north showed the highest DNA damage ($p<0.007$) when compared to those collected in the center and south. Both size fractions of concentrated PM collected in November showed significant regional and size differences: coarse PM from the north showed a significantly stronger effect ($p<0.0001$) than the fine fraction, while particles from the center showed no significant difference.

The comparison of DNA damage of cells exposed to PM from the same region and the same fraction collected in May and November showed significant temporal differences ($p < 0.0001$). These results are in accordance with previous reports where the higher comet length was observed in mouse fibroblasts exposed to PM_{10} from the north (Alfaro-Moreno et al., 2002). Our data showing a similar behavior in terms of the higher DNA damage and transition metal load in PM is in agreement with Knaapen et al. (2002) who have suggested that transition metals present in PM contribute to a higher DNA damage.

In conclusion, the data presented in this study contribute to the hypothesis that ambient particle size fraction, chemical composition and emission source are influenced by temporal and regional variations and have some bearing on their biological effects. The coarse fraction from the north had a higher metal content, more noticeable during November, than the fine fraction and higher levels of bacteria, fungi and endotoxin. Yet a different tendency was observed for redox activity and carbon content, where the higher activity and content were observed for the fine fraction. Both PM fractions from the north showed a higher ability to induce apoptotic cell death and DNA damage. The VACES system is an effective instrument to collect PM. However, a more detailed examination of chemical components should be performed to explain the differences between coarse and fine PM chemical characteristics and their repercussions on their toxic effects.

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