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Monitoring Urban Air Particulate Matter (Fractions PM 2.5 and PM 10) Genotoxicity by Plant Systems and Human Cells In Vitro: A Comparative Analysis

Anna Poma,[1]* Lorenzo Arrizza,[2] Pietro Picozzi,[1] and Laura Spanò[1]

[1] *Department of Basic and Applied Biology, Faculty of Sciences, University of L'Aquila, Coppito, L'Aquila, Italy*

[2] *Department of Physics, Faculty of Sciences, University of L'Aquila, Coppito, L'Aquila, Italy*

Increased incidence of mortality and sickness due to cardiopulmonary complications has been associated with elevated levels of urban air particles (UAP), with an aerodynamic diameter of 10 μm (PM 10) and 2.5 μm (PM 2.5). In the present report alternative plant systems and human cells in vitro are associated with human hazard and genotoxic risk assessment of UAP. The genotoxic activities associated with the coarse (PM 10) and the fine fraction (PM 2.5) of airborne particulates have been analyzed by evaluating micronuclei induction and/or sister-chromatid exchange (SCE) using in vitro models of *Daucus carota* and HS 27 human fibroblast cell suspensions and *Zea mays* root meristems. Results show variability in the response of the test systems and indicate that the mutagenicity trend in both plant and human cell cultures was directly correlated to the concentration of carbon-rich particles in the fraction of the PM 2.5 airborne particulates. Moreover, in plant tissues, the frequency of micronuclei and SCE was related to an enhancement of the specific activity of the stress-related enzyme peroxidase. *Teratogenesis Carcinog. Mutagen. 22:271–284, 2002.* © 2002 Wiley-Liss, Inc.

Key words: airborne particulates (PM 10; PM 2.5); *Daucus carota*; human fibroblasts; micronuclei; sister chromatid exchanges; *Zea mays*

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*Correspondence to: Anna Poma, Department of Basic and Applied Biology, Faculty of Sciences, University of L'Aquila, Via Vetoio, Coppito, L'Aquila, Italy. E-mail: poma@univaq.it

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INTRODUCTION

Many analytical studies have shown that urban air, particularly in high traffic areas, contains mutagenic and carcinogenic substances, including organic compounds, such as benzo(a)pyrene and benzene, or inorganic compounds, such as nickel and chromium, and radionuclides [1]. Experimental studies have shown that extracts of urban air particulates can induce cancer in animals and are mutagenic in bacteria and mammalian cells [2]. In recent years the increase of PM10 (diameter 10 μm) and PM 2.5 (diameter 2.5 μm) particulate matter concentrations have been associated with an increased number/frequency of cardiorespiratory hospital admissions, cardiorespiratory mortality, and respiratory-related emergency department visits. These effects are observable with PM10 concentrations in the range of the quality air standards [3]. However it is not known which aspects of urban air particles (mass, size, shape, composition, or a combination of at least these three aspects, might be contributing to the observed effects on health. A recent theory is that transition metals, present as surface contaminants, can promote the production of free radicals which may cause the formation of reactive oxygen species as superoxide anions, hydroxyl and hydroperoxy radicals [4]. Some researchers propose that the ultrafine particles act as vehicles for sulphuric acid, initiating focal lung damage, when they impact the epithelial surfaces [5]. The monitoring of urban air for genotoxic and mutagenic micropollutants, in addition to conventional pollutants, is receiving increasing consideration with the aim of evaluating health risks in urban areas [6].

The aim of the present research is to describe, from a physico-chemical viewpoint, the particles constituting the PM 10 and PM 2.5 in an urban area (the town center of L'Aquila) with high density vehicular traffic and to compare the genotoxic effects induced by urban airborne particulate mixtures (PM 10, coarse, and PM 2.5, fine particles) on plant and human cells in vitro. Cytological evaluation of the poisoning effects of air particulate on plant systems has scarcely been reported [7]; in order to further the understanding of the genotoxic effects of air particulate on crops, the micronucleus (MN) assay and the sister chromatid exchange (SCE) tests were performed on root tips of *Zea mays* cultured in vitro in comparison to HS 27 human fibroblasts. In addition, biochemical alterations suggestive of air particulate induced stress were checked in *Zea mays* roots in vitro by monitoring peroxidase activity. The effects of the particulate samples on *Daucus carota* cultured cells were studied by evaluating micronuclei and the mitotic indices.

MATERIAL AND METHODS

Airborne Particulates: Sample Collection, Extraction and Characterization

Airborne particles were collected by an eight-stage cascade impactor (Andersen particle fractionating sampler) with a preseparator that eliminates particles with aerodynamic diameter > 10 μm. The flow rate of the sampler was 28.317 l/min. The sampler separates the particulate into eight granulometric fractions within the 10–0.4 μm aerodynamic diameter range.

In this work we studied two fractions: the particulate with aerodynamic diameter ranging from 10 to 2.5 μm (coarse particles) and the particulate with aerodynamic diameter ranging from 2.5 to 0.4 μm (fine particles).

After each sampling, the stainless disks of the cascade impactor were put in an ultra-sound bath with ethyl alcohol to remove the particles collected. Part of the suspension obtained was filtered on polycarbonate membranes, with 0.4 μm pore size. Portion of filters were mounted on SEM stubs and then coated with a thin carbon film. The samples for biological determinations were evaporated with a rotary evaporator and dissolved in dimethyl sulfoxide (DMSO) to obtain a concentration of 50 m^3 air volume equivalent/ml extract solution; in cell culture experiments air volume of collection of particulate was tested as 5 and 10 m^3/ml culture medium.

Single-Particle Analysis by Automated Scanning Electron Microscopy

The analysis of the particles was performed by a SEM/EDX Philips XL30 equipped with a thin-window EDAX DX4 system for X-ray microanalysis by energy dispersion spectrometry. For every stage of the cascade impactor particles were automatically detected by an increase in the particle's back-scattered electron (BSE) video signal above a preset video threshold.

For each particle, we determined the average diameter, the perimeter, the area, and the roundness. After the size analysis was completed, the electron beam was continuously rastered in a square with side equal to the particle's average diameter and an X-ray spectrum (0–25 keV) was acquired for 40 sec. The intensities of the characteristic X-ray lines were converted into corresponding atomic concentration by a standardless ZAF correction method [the backscattering and the power stopping depend on the atomic number (Z), the X-ray absorption (A), and the fluorescence (F)].

Numerical Data Analysis

The data set was analyzed using the Hierarchical Cluster Analysis (HCA) to classify the particles into groups (clusters) with chemically similar composition and to recognize the abundance of major particulate components in each granulometric fraction according to reference [8]. The particles atomic concentration of the constituent chemical elements was determined. We adopted the "squared Euclidean distance" as the distance measure between particles. The result of this classification algorithm is a hierarchical tree in which the particle clusters are organized on the basis of growing distances. For each stage (granulometric fraction) of the cascade impactor the abundance of each particle cluster was calculated.

Plant Cell Systems

***Daucus carota* cultures.** *Daucus carota* (carrot) long-term cultures were grown in liquid B5 medium supplemented with sucrose 20 g/l, 2,4-D (2,4-dichlorophenoxyacetic acid) 0.5 mg/l, and BPA (N-benzyl-9-2-tetrahydropyranyl adenine) 0.25 mg/l as reported by De Lorenzo et al. [9]. For toxicity tests the cells were incubated with complete medium supplemented with appropriate volumes of particulate solutions at various concentrations; negative controls were incubated with the same volume of buffer; samples to which $CdCl_2$ (0.1 M final concentration) were used as positive controls. Treatments were performed at 26°C for 2–5 days; and two repeat experiments were done. Cell cultures growth was monitored by increase in packed cell volume. Cells were observed by phase-contrast microscopy to detect

cell structure changes and then fixed in 3 parts 95% ethanol:1 part glacial acetic acid for 24 h and hydrolyzed in 1 M hydrochloric acid at 60°C for 5 min, and then stained and squashed in a solution of acetocarmine stain on a microslide. About 4,000 cells were scored for mitotic index and micronuclei; mitotic abnormalities were observed from dividing cells.

The *Zea mays* Root Tip-Cell Micronucleus Assay

Equal-sized seeds of *Zea mays* hybrid F1 "Merit" (F.lli Ingegnoli, Milan) were chosen and germinated in distilled water. When the radicals elongated to 2–3 mm, appropriate volumes of particulate samples at various concentrations were added to 100 mm diameter culture dishes; negative controls were incubated with the same volume of buffer; and $CdCl_2$ (final concentration 0.1 M) treated samples were used as positive controls. Treatments were performed at 26°C for 48 h. The exposed root tips were fixed in 3 parts 95% ethanol:1 part glacial acetic acid for 24 hr and washed in distilled water. These fixed materials were hydrolyzed in 1 M HCl and 95% ethanol (1:1) at R.T. for 2 to 3 min and washed thoroughly in distilled water before storage. Temporary slides were prepared by staining the dispersed cells from the 2 mm tips of the root with acetocarmine on a clean slide and squashed under a coverglass. The micronuclei (MN) frequencies were scored from each of the five slides (1,000 cells per slide) of an experimental group. Student's t-test was applied to determine the significance of the difference between treated and control MN frequencies.

SCE determinations on *Zea mays* roots tip-cells. The experiments were carried out with primary root tip cells of *Zea mays* according to [10]. Cells from primary roots tip, 1–2 mm long, were incubated for 20 h (approximately one cell cycle) in a solution of 10^{-4} M bromodeoxyuridine (BrdU), 10^{-8} M fluorodeoxyuridine (FdU), and 10^{-6} M uridine (UrD). Thereafter the treatment solution was renewed and there was a change of medium (fresh BrdU added). The roots were treated for another 20 h in order to ensure BrdU incorporation throughout two consecutive cycles of replication. The plant tissues remained in 0.05% colchicine for 2 h before fixation. They were fixed overnight in ethanol-acetic acid (3:1) at 4°C. Before fixation the whole procedure was performed in the dark. The preparation of slides was according to [10].

Exposure schedule for test particulates and analysis of SCE. Following the first round of treatments with BrdU, FdU, and UrD for 20 h, the growing root meristems were exposed to the coarse and fine particulates at various concentrations (5 and 10 m^3/ml) for 48 h in the presence of dT and Urd. Positive controls were performed with 10 μM maleic hydrazide. Subsequently the roots were treated with colchicine, washed, excised, and fixed for analysis of SCE following as above described. Parallel tap water controls were included and handled alike for each set of treatments. At least 20 metaphase spreads from four or more meristems for each concentration were analyzed. Increase in the frequency of SCE was tested statistically using one tailed t-test.

Peroxidase Activity

Protein content of *Zea mays* roots extracts were determined according to the method of Bradford [11] using bovine serum albumin (BSA), Sigma, as the protein standard. Enzymatic activity was determined according to [12] and expressed as specific activity (U/mg^{-1} of protein).

HS 27 Human Skin Fibroblast Cultures

HS 27 human skin fibroblasts (a kind gift of Prof. E. Alesse, Department of Experimental Medicine, University of L'Aquila) were maintained in RPMI 1640 medium containing 10% fetal calf serum, 2 mM L-glutamine, 20 mM Hepes, 100 IU/ml penicillin, and 100 μg/ml streptomycin. The cultures were incubated under 5% CO_2 moist air at a humidity of 80% and a temperature of 37°C. Cell detachment was carried out with trypsin-EDTA (0.5% of porcine trypsin and 0.2% EDTA).

Micronucleus assay in HS 27 fibroblasts. HS 27 fibroblasts were incubated with complete medium supplemented with appropriate volumes of particulate samples (coarse and fine) at various concentrations (5 and 10 m^3/ml); negative controls were incubated with the same volume of buffer and positive controls were performed with lead acetate (Pb) at 0.2, 2, and 200 ng/ml. Treatments were performed for 44 h. Cells were then assayed for micronucleus production according to the procedure by Tawn and Holdsworth [13]. Cytochalasin B (Cyt B) (Sigma Chemical Co., St. Louis, MO), at a final concentration of 6 μg/ml, was added to 44 h cultures in fresh complete medium containing the particulate extracts and/or positive controls. Cells were harvested 28 h after Cyt B addition (total culture time being 72 h). Three parallel cultures were used for each experiment and three independent determinations were carried out for each type of particulate. After treatment, fibroblasts were collected by low-speed centrifugation (350 g for 10 min), washed twice in Dulbecco's PBS and finally resuspended in 1 ml fixative (3:1 methanol: acetic acid); fixed cells were smeared on clean glass slides, air dried, and stained with Giemsa for 3 min. Three thousand binucleated cells per treatment group (1,000 from each culture) were scored to detect micronucleus formation at × 1,000 magnification by light microscopy.

Sister-chromatid exchange assay in HS 27 fibroblasts. Cell cultures were incubated in complete medium supplemented with BrdU (10 μg/ml final concentration) in the dark for 2 days; there was a change of medium (fresh BrdU added) before particulates at various concentrations were added. HS 27 fibroblasts were cultured for 24 h in the medium supplemented with 5 and 10 m^3/ml fine and coarse particulates. Colcemid was then added, 4 h before harvesting. Hypotonic treatment and fixation of the cells were performed according to the standard techniques. SCE staining was carried out according to reference [13]. After staining with Hoechst for 15 min, the slides were washed in distilled water, mounted in 2 × SSC and exposed to an UV lamp (366 nm) for 1 h in a humid chamber (exposure to light selectively degrades the BrdU highly substituted chromatid). Following incubation at 60°C in 2 × SSC for another 2 h, they were stained with 2% Giemsa solution for 10 min and observed by conventional light microscopy. More then 50 metaphases for each culture were scored. Results were analyzed by the Student's t test to compare SCE/chromosome mean values of different cultures.

HS 27 fibroblasts cytotoxicity test. Cell viability of treated cells was measured by counting cells (100–200) after trypan blue exclusion in a Thoma-Zeiss chamber.

RESULTS

Hierarchical Cluster Analysis

From the results of the Hierarchical Cluster Analysis (), seven principal particle types (clusters) were identified in the PM10 of the studied area: C-rich particles (C > 80%), Ca carbonates, silica, Ca sulfates, silicates, Fe-rich particles (Fe > 50%), metals, and metallic compounds (Al, Ti, Zn, Cr, Ni, Cu, and Pb). This cluster shows the typical granulometric pattern of particulate from soil erosion. The Ca carbonates, Ca sulfates, and Fe-rich particles are more frequent in the coarse granulometric fractions (diameter ranging from 2.5 to 10 μm). The C-rich particles, which, in the studied area, derive from vehicular traffic, prevail in the fine granulometric fractions (<2.5 μm). In the ultrafine granulometric fractions (<1.0 μm), the abundance of this particle type is greater than 90%. The C-rich particles () often carry strong acids, such as H_2SO_4 and HCl, adsorbed on the surface, evidenced as zones with high concentrations of S and Cl anions. The abundance of the particles carrying strong acids increases as particle diameter decreases. In the ultrafine C-rich particles more than 60% of total show these acid deposits.

Micronuclei (MN) and Sister Chromatid Exchange (SCE) Tests on Plant Cells Systems

The mitotic index reflects the frequency of cell division and rate of growth. In it can be seen that with duration of the treatment, both for fine and coarse treated cells, there was a significant decrease in the mitotic index. PM 2.5 induced micronuclei production in *Daucus carota* cells at concentration of 10 m^3/ml (2 and 5 days exposure period); PM 10 showed a lower micronuclei induction (10 m^3/ml, 5 days cells exposure period). The test was validated by treating cells with $CdCl_2$ because it is much more easily taken up by plants than other heavy metals [14].

TABLE I. Hierarchial Cluster Analysis (Coarse and Fine, Year 2000 Samples)

		C-rich particles (%)	Carbonates (%)	Sulphates (%)	Silica (%)	Silicates (%)	Fe-rich particles (%)	Metals (%)
3–10 February	Coarse	27.40	44.10	4.30	3.70	13.90	5.30	1.30
3–10 February	Fine	86.50	2.30	3.40	1.40	4.20	1.10	1.10
9–16 March	Coarse	26.00	34.90	7.30	8.60	17.80	3.20	2.20
9–16 March	Fine	92.10	0.80	1.00	1.30	3.20	0.80	0.80
26 April–3 May	Coarse	26.30	30.90	7.00	7.90	24.20	2.80	0.90
26 April–3 May	Fine	76.70	3.30	2.70	6.90	8.30	1.50	0.60
6–13 June	Coarse	2.00	68.30	0.90	3.00	22.80	1.20	1.80
6–13 June	Fine	80.60	6.70	5.30	3.70	2.00	1.00	0.70
12–19 July	Coarse	18.70	43.20	4.20	5.50	21.20	2.30	4.90
12–19 July	Fine	78.20	6.70	5.50	0.90	6.40	1.40	0.90
25 Sep–3 Oct	Coarse	10.40	57.50	1.60	3.70	21.60	3.50	1.70
25 Sep–3 Oct	Fine	77.80	4.20	5.80	2.30	6.10	1.90	1.90

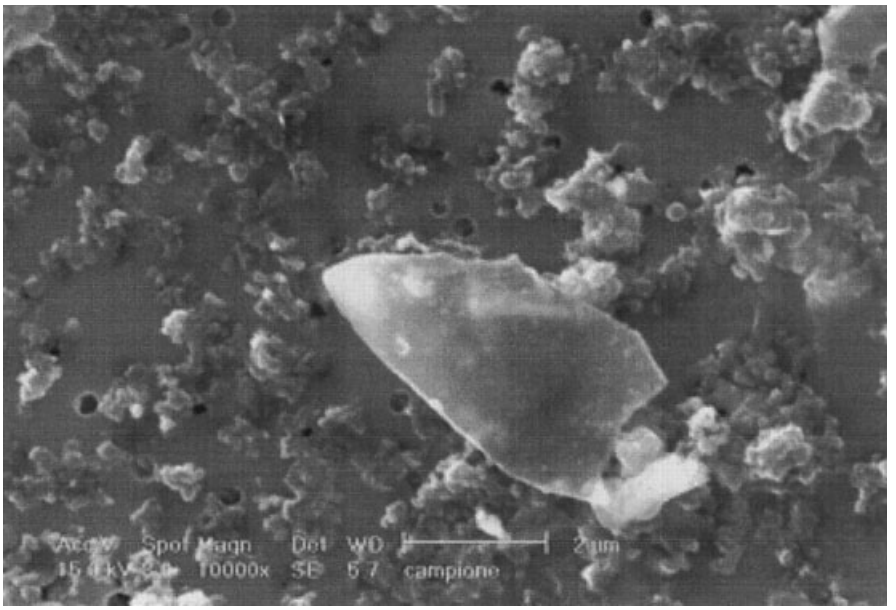

Fig. 1. C-rich particles with acid deposits (the brighter zones on the particle).

By using *Zea mays* root tips, a slight inhibition of root growth was recorded (data not shown); moreover the micronuclei test (see) evidenced a significant induction of micronuclei by fine particulates (5,10 m^3/ml) at the tested conditions. SCE induced by PM 10 and 2.5 were also evaluated in *Zea mays* primary root tips (). A dose-dependent increase of sister chromatid exchanges frequencies was observed for both fractions, being statistically significant at concentrations equivalent to airborne fine particulates of 10 m^3/ml. A lower effect was associated with coarse particulate treatments.

Peroxidase Activity in *Zea mays* In Vitro UAP-Treated Roots

Biochemical alterations suggestive of air pollution stress, even in the absence of visible genotoxicity symptoms, were analyzed. Peroxidase activity have been used to monitor stress induced by atmospheric pollution [15]. summarizes the data on the peroxidase activity in Zea mays roots extracts after 48 h treatment with particulate. $CdCl_2$ (10^{-1}–10^{-4} M) treated roots were used as positive controls for assay validation. Samples treated with fine particulate exhibited enhanced root peroxidase activity in comparison to controls. The results support the idea that the peroxidase could constitute an important detoxification system involved in a rapid adaptation of *Zea mays* exposed to air particulate; in fact a correlation exists between the frequency of both SCE and micronuclei and peroxidase activity in the exposed plant tissues. A significant rise of the enzyme activity in the intracellular liquid was observed in the samples treated with the PM 2.5 particulate (5 m^3/ml) compared with the PM 10 (5 m^3/ml) ones; the activity appeared to increase compared with the control also in the PM 10 (10 m^3/ml) treated samples but with less enhancement.

TABLE II. Mitotic Indices and Frequency of Micronuclei Observed in *Daucus carota* Cultured Cells 2 and 5 Days Treated With Different Concentrations of Airborne Particulates (Fine and Coarse, 2000)

		February		March		April		June		July		September	
Time (days)	Treatment	Mitotic index (%)	Micronuclei/ 1,000 cells[a,b]	Mitotic index (%)	Micronuclei/ 1,000 cells[a,b]	Mitotic index (%)	Micronuclei/ 1,000 cells[a,b]	Mitotic index (%)	Micronuclei/ 1,000 cells[a,b]	Mitotic index (%)	Micronuclei/ 1,000 cells[a,b]	Mitotic index (%)	Micronuclei/ 1,000 cells[a,b]
2	Fine (5 m^3/ml)	7.0±1.0	0	6.7±0.7	0	7.1±0.9	0	6.5±1.1	0	7.0±1.5	0	7.5±1.7	0
	Fine (10 m^3/ml)	7.0±1.5[d]	10±1[c]	6.7±1.7[d]	11±1[c]	7.1±0.7[d]	12±1[c]	6.5±1.7[d]	10±1[c]	7.0±1.5[d]	14±1[c]	7.5±1.5[d]	13±1[c]
	Coarse (5 m^3/ml)	9.0±1.5	0	9.1±1.7	0	8.9±1.5	0	8.7±1.8	0	8.9±1.4	0	9.2±1.6	0
	Coarse (10 m^3/ml)	9.0±1.2	0	9.1±1.5	0	8.9±1.7	0	8.7±1.6	0	8.9±1.3	0	9.2±1.7	0
	Control	11.0±1.0	0	11.0±1	0	11.5±1.0	0	11.0±1.0	0	11.5±1.2	0	12.0±0.7	0
	Positive control (Cd 10^{-1} M)	2.0±0.1[d]	30±1.5[c]	2.0±0.1[d]	25±1[c]	2.0±0.7[d]	28±1[c]	2.0±0.2[d]	22±1[c]	2.5±0.7[d]	23±1[c]	2.0±0.6[d]	31±1[c]
5	Fine (5 m^3/ml)	1.0±0.1	0	6.5±1.2	0	6.9±1.2	0	6.4±1.5	0	6.8	0	7.3±1.7[d]	0
	Fine (10 m^3/ml)	1.0±0.1[d]	20±1.5[c]	6.5±1.7[d]	23±1.5[c]	6.9±1.1[d]	24±1[c]	6.4±1.7[d]	20±1[c]	6.8±1.0[d]	22±1[c]	7.3±1.5[d]	20±1[c]
	Coarse (5 m^3/ml)	8.0±1.1	0	8.1±1.4	0	7.9±1.6	0	7.8±1.6	0	7.9	0	8.1±1.6	0
	Coarse (10 m^3/ml)	5.0±0.7[d]	11±1[c]	5.0±1.3[d]	13±1[c]	4.9±1.2[d]	0	4.8±1.1[d]	13±1[c]	4.9±1.4[d]	12±1[c]	5.1±1.4[d]	14±1[c]
	Control	9.0	0	1.0±1.0	0	9.0±1.1	0	9.0±1.3	0	9.5	0	10.0±1.1	0
	Positive control (Cd 10^{-1} M)	0.0	40±2[c]	0.0	50±2[c]	0.0	30±2[c]	0.0	30±1[c]	0.3±0.1[d]	40±2[c]	0.0	30±2[c]

[a]Range of micronuclei per cell: 1.

[b]Values are means±SEM from six independent samples.

[c,d]Mean value significantly different, Student's *t*-test; ([c]$P<0.001$; [d]$P<0.01$) for values compared with the control.

TABLE III. Frequency of Micronuclei in Root Tip Cells of *Zea mays* in the Presence of Airborne Particulates (Coarse and Fine, 48 h In Vitro Exposure Period)

Treatment	Period of samples collection (year 2000)	Micronuclei number/ 1,000 cells[a,b]
Positive control (CD 10^{-1} M)		36 ± 1.9
Control		0.0
Coarse 5 m^3/ml	February	0.0
Fine 5 m^3/ml	February	1.1 ± 1.6[c]
Coarse 5 m^3/ml	March	0.0
Fine 5 m^3/ml	March	10 ± 1.6[c]
Coarse 5 m^3/ml	April	0.0
Fine 5 m^3/ml	April	12 ± 0.5[c]
Coarse 5 m^3/ml	June	0.0
Fine 5 m^3/ml	June	13 ± 1.2[c]
Coarse 5 m^3/ml	July	0.0
Fine 5 m^3/ml	July	10 ± 1.2[c]
Coarse 5 m^3/ml	September	0.0
Fine 5 m^3/ml	September	14 ± 1.2[c]
Coarse 10 m^3/ml	February	10 ± 1.0[c]
Fine 10 m^3/ml	February	22 ± 1.1[c]
Coarse 10 m^3/ml	March	11 ± 1.3[c]
Fine 10 m^3/ml	March	23 ± 1.0[c]
Coarse 10 m^3/ml	April	0.0
Fine 10 m^3/ml	April	24 ± 1.0[c]
Coarse 10 m^3/ml	June	0.0
Fine 10 m^3/ml	June	22 ± 1.5[c]
Coarse 10 m^3/ml	July	0.0
Fine 10 m^3/ml	July	25 ± 1.0[c]
Coarse 10 m^3/ml	September	10 ± 1.3[c]
Fine 10 m^3/ml	September	24 ± 1.2[c]

[a]Range of micronuclei per cell: 1.
[b]Values are means $\pm$ SEM from six independent samples.
[c]Mean value significantly different, Student's *t*-test; ($P < 0.001$) for values compared with the control.

Micronuclei (MN) Test and SCE Determinations in HS 27 Fibroblasts Cells Exposed to Urban Particulates

The cytotoxic effect of fine and course particulate to human HS 27 fibroblasts cells in vitro was tested; cells were analyzed by means of the trypan blue exclusion method for evaluating their viability. In order to verify the effect of fine and coarse UAP on the stability of chromosomal DNA of treated cells, we performed a test for the quantitation of micronuclei induction. Changes in the nuclear morphology accompanied by the appearance of micronuclei in the cytoplasm of treated cells were observed (data not shown). As reported in , both fine and coarse particulate induced micronuclei and SCE formation in treated fibroblasts at the doses of 5 and 10 m^3/ml. The frequency of micronuclei was higher in the fine particulate-treated fibroblasts in respect to the coarse treated one; this fraction (PM 2.5) exerted a stronger genotoxic activity as measured by SCE induction.

The tests were validated with lead acetate at doses (2 and 200 ng/ml) comparable with Pb plasma levels in the general population chronically exposed [1].

TABLE IV. Induction of Sister Chromatid Exchange (SCE) in Root Tip Cells of *Zea mays* in the Presence of Particulates (Coarse and Fine, 48 h In Vitro Exposure Period)

Treatment	Period of samples collection (year 2000)	Counted chromosomes	SCE/ chromosome
Positive control (10.0 μM maleic hydrazide)		548	6.1 ± 0.02[a]
Control		500	2.0 ± 0.05
Coarse 5 m^3/ml	February	580	4.0 ± 0.5
Fine 5 m^3/ml	February	598	5.5 ± 0.6[a]
Coarse 5 m^3/ml	March	530	4.4 ± 0.5
Fine 5 m^3/ml	March	540	5.4 ± 0.6[a]
Coarse 5 m^3/ml	April	500	4.8 ± 0.6
Fine 5 m^3/ml	April	500	5.0 ± 0.5[a]
Coarse 5 m^3/ml	June	580	4.4 ± 0.1
Fine 5 m^3/ml	June	550	4.6 ± 0.2
Coarse 5 m^3/ml	July	502	4.8 ± 0.1
Fine 5 m^3/ml	July	500	5.0 ± 0.2[a]
Coarse 5 m^3/ml	September	500	4.6 ± 0.1
Fine 5 m^3/ml	September	504	5.0 ± 0.2[a]
Coarse 10 m^3/ml	February	545	5.9 ± 0.2[a]
Fine 10 m^3/ml	February	567	7.0 ± 0.1[a]
Coarse 10 m^3/ml	March	500	5.6 ± 0.3[a]
Fine 10 m^3/ml	March	500	7.2 ± 0.3[a]
Coarse 10 m^3/ml	April	570	5.8 ± 0.1[a]
Fine 10 m^3/ml	April	546	6.4 ± 0.3[a]
Coarse 10 m^3/ml	June	564	4.6 ± 0.2
Fine 10 m^3/ml	June	500	5.8 ± 0.1[a]
Coarse 10 m^3/ml	July	510	5.4 ± 0.1[a]
Fine 10 m^3/ml	July	498	5.8 ± 0.2[a]
Coarse 10 m^3/ml	September	500	5.6 ± 0.3[a]
Fine 10 m^3/ml	September	470	7.0 ± 0.2[a]

[a]Mean value significantly different, Student's *t*-test; ($P < 0.001$) for values compared with the negative control.

DISCUSSION

Seven principal particle types (clusters) were identified in the PM10 of the studied area: C-rich particles (C > 80%), silica, Ca carbonates, Ca sulfates, Fe-rich particles, silicates, metals, and metallic compounds (Al, Ti, Zn, Cr, Ni, Cu, and Pb). The C-rich particles, which, in the studied area, derive from vehicular traffic, prevail in the fine granulometric fractions. It has been established that polycyclic aromatic hydrocarbons in urban atmosphere are contained mostly in the fine particles that were found in the intrapulmonary deposition [16–20]. Interestingly enough, most of the studies that have focused on the mutagenic effects of hydrocarbons adsorbed in the particulate matters of urban air require the use of organic solvents for their extraction. Since there is no solvent-extraction process in the pulmonary environment, such studies would create an artificial situation that could mislead the interpretation of the genotoxic effects of urban particles. Moreover the physical characteristics (number, sizes, shape, and aggregation properties) contribute towards ill health. We highlight the importance of characterization of PM10 and PM 2.5 individual particles by Hierarchical Cluster Analysis in the monitoring of urban air

TABLE V. Variation of Peroxidase Activity From *Zea mays* Root Samples Exposed to Air Particulate Matter (48 h In Vitro Exposure)

Treatment	Period of samples collection (year 2000)	Peroxidase activity μmol pyrogallolo/min/mg protein extract
Positive control (Cd 10^{-4} M)		50.82 ± 1.10^{a}
Positive control (Cd 10^{-4} M)		11.49 ± 0.9^{a}
Control		6.57 ± 1.08
Coarse 5 m^3/ml	February	27.61 ± 0.88^{b}
Fine 5 m^3/ml	February	50.57 ± 0.99^{b}
Coarse 5 m^3/ml	March	27.41 ± 1.03^{b}
Fine 5 m^3/ml	March	47.51 ± 1.08^{b}
Coarse 5 m^3/ml	April	26.75 ± 1.12^{b}
Fine 5 m^3/ml	April	47.56 ± 0.98^{b}
Coarse 5 m^3/ml	June	26.37 ± 0.75
Fine 5 m^3/ml	June	44.13 ± 1.01^{b}
Coarse 5 m^3/ml	July	26.84 ± 0.93^{b}
Fine 5 m^3/ml	July	44.95 ± 0.98^{b}
Coarse 5 m^3/ml	September	27.13 ± 0.67^{b}
Fine 5 m^3/ml	September	47.15 ± 0.99^{b}
Coarse 10 m^3/ml	February	27.11 ± 0.58^{b}
Fine 10 m^3/ml	February	25.57 ± 0.99^{b}
Coarse 10 m^3/ml	March	27.31 ± 1.00^{b}
Fine 10 m^3/ml	March	23.51 ± 1.00^{b}
Coarse 10 m^3/ml	April	26.75 ± 1.12^{b}
Fine 10 m^3/ml	April	23.46 ± 0.98^{b}
Coarse 10 m^3/ml	June	22.89 ± 0.65
Fine 10 m^3/ml	June	20.10 ± 1.00^{b}
Coarse 10 m^3/ml	July	24.84 ± 0.90^{b}
Fine 10 m^3/ml	July	21.35 ± 0.98^{b}
Coarse 10 m^3/ml	September	27.14 ± 0.66^{b}
Fine 10 m^3/ml	September	21.11 ± 0.89^{b}

Values are means $\pm$ SEM from at least five independent determinations.
[a]Mean value significantly different, Student's *t*-test; ($P < 0.025$) for values compared with the control.
[b]Mean value significantly different, Student's *t*-test; ($P < 0.005$) for values compared with the control.

for genotoxic and mutagenic micropollutants, in addition to conventional pollutants with the aim of evaluating health risks in urban areas; in fact a positive correlation was established between PM 2.5 C-rich particles percentages and genotoxicity.

Results presented here extend the possibility of using in vitro tests (alternative to microrganisms) on plant and human cells for testing genotoxicity of urban air particulate matter. Our data on *Daucus carota* cultured cells and *Zea mays* root tip cells (alternative to *Vicia* and/or *Tradescantia*) indicate that the tests used can be useful in estimation of bioavailability and genotoxic damage due to environmental air pollutants in higher plants. The more significant genotoxic activity of the PM 2.5 fraction presented in this study both on plant and human cells is in good agreement with the report of Hornberg et al. [21]. The present study highlights evidence for micronuclei and SCE induction by fine and coarse particulate and the human HS 27 skin fibroblasts cell line offers a reliable and sensitive in vitro model for genotoxicity testing of PM 10 and PM 2.5; in parallel, the capability of plant systems in vitro and also in vivo to show the response to environmental air pollutants/particulate largely

TABLE VI. Frequency of Micronuclei in Cultured HS 27 Human Fibroblasts Cells Exposed to Airborne Particulates

	Number of micronuclei/1,000 binucleated cells[a,b]					
Treatment	February	March	April	June	July	September
Fine 5 m^3/ml	31.4±1.8[c]	23.2±2.0	83.4±1.5[c]	28.3±1.0[d]	44.2±1.1[d]	32.7±1.2[d]
Fine 10 m^3/ml	50.3±2.0[c]	43.4±1.9[c]	90.1±2.0[c]	46.2±1.5[d]	80.3±1.9[e]	49.2±1.4[d]
Coarse 5 m^3/ml	44.5±2.9[c]	24.2±1.8	68.2±2.0[c]	29.2±0.9[d]	51.2±1.1[c]	37.3±0.9[d]
Coarse 10 m^3/ml	46.2±2.8[c]	36.5±1.7[c]	82.3±1.9[c]	44.2±1.3[d]	77.3±1.5[e]	47.1±1.1[c]
Control	16.2±0.5	15.2±0.5	15.5±0.5	14.5±0.5	16.8±0.5	16.9±0.5
Positive control						
2 ng/ml lead acetate	68±2.0[c]	70.1±2.0[c]	68.2±2.2[c]	68.1±2.0[c]	69.4±2.2[c]	67.6±2.2[c]
200 ng/ml lead acetate	97.2±1.5[c]	90.4±1.7[c]	100.1±2.5[c]	97.5±1.7[c]	95.2±1.7[c]	100.2±2.5[c]

[a]Range of micronuclei per cell: 1.
[b]Values are means±SEM from three independent cultures.
Values significantly different from the control: [c]$P<0.0125$; [d]$P<0.025$; [e]$P<0.010$.

TABLE VII. Dose Related Induction of Sister Chromatid Exchange in Human Fibroblasts (Line HS 27) in the Presence of Airborne Particulates (Fractions Coarse and Fine Collected in the Year 2000)

Treatment	Dose	SCE/chromosome	RI[d]
Particulate February fine	5 m^3/ml	0.91±0.03[a]	1.26
Particulate February coarse	10 m^3/ml	0.72±0.02[a]	1.25
Particulate March fine	5 m^3/ml	0.82±0.02[a]	1.04
Particulate March coarse	10 m^3/ml	0.61±0.03	1.45
Particulate April fine	5 m^3/ml	0.80±0.02[b]	1.20
Particulate April coarse	10 m^3/ml	0.66±0.02[b]	1.20
Particulate June fine	5 m^3/ml	0.80±0.04[c]	1.23
Particulate June coarse	10 m^3/ml	0.60±0.03	1.30
Particulate July fine	5 m^3/ml	0.75±0.03[c]	1.23
Particulate July coarse	10 m^3/ml	0.30±0.07	1.52
Particulate September fine	5 m^3/ml	0.91±0.03[a]	1.11
Particulate September coarse	10 m^3/ml	0.82±0.02[b]	1.10
control		0.44±0.02	1.46
Positive control (lead acetate)	2 ng/ml	0.70±0.04[a]	1.21
	200 ng/ml	0.98±0.02[b]	1.11

Values significantly different from the control: [a]$P<0.025$; [b]$P<0.010$; [c]$P<0.05$.
[d]Replication index (RI): 1 M_1 + 2 M_2 + 3 M_3 + 4 M_4/100.

counterbalances a lowering in sensitivity [22,23]. However, a recent survey of studies has shown that excellent correlations exist between the frequency of both chromosomal abnormalities and C-mitoses in plant and animal systems [24]. We conclude that the use of higher plant (in vitro cultured cells and roots systems), human cells systems (in vitro skin fibroblasts), and particulate Hierarchical Cluster Analysis (HCA) could give further information regarding environmental air particulate genotoxic load.

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