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Diesel emissions significantly influence composition and mutagenicity of ambient particles: a case study in São Paulo, Brazil ☆

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Abstract

In 2003, a bus strike paralyzed the fleet of buses in São Paulo, Brazil during 3 days, from 6 to 8 of April, the complete interruption of services being achieved on the 7th. We evaluated the effect of the absence of this source of pollution on the composition, mutagenicity, and toxicity of the fine particulate material collected during this period. Particles were sampled in glass fiber filters on days 7 and 15 of April of 2003 (strike and nonstrike days, respectively), using a high-volume sampler. Trace element determinations (As, Br, Co, Cl, Fe, La, Mn, Sb, Sc, and Th) of particulate material samples were carried out by neutron activation analysis. Sulfur determination was done by X-ray fluorescence analysis. The ratio between nonstrike/strike concentrations of hydrocarbons associated with automotive emissions (benzene, toluene, ethyl-benzene, and xylenes; BTEX) was determined by gas chromatography/mass spectrometry. Mutagenesis of testing solutions was determined by means of the *Tradescantia* micronucleus assay in early tetrads of *Tradescantia pallida*. The inhibition of mitosis of the cells of the primary meristema of the root tips of *Allium cepa* was used as an index of the toxicity. Fine particle trace element contents were lower during the strike. The concentrations of sulfur and BTEX were 50% and 39.3% lower, respectively, on the strike day. A significant ($P = 0.038$) reduction of micronuclei induced by fine particles sampled during the strike was observed. No effect of the strike on toxicity was detected. These results indicate that a program aiming to reduce emissions of the bus fleet in our town may impact positively the air quality by reducing the mutagenic potential of ambient particles.

Keywords: Diesel emissions; Mutagenesis; Air pollution; Environmental toxicology; Ambient particles

1. Introduction

The relationship between air pollution and cancer has been consistently demonstrated by epidemiological studies (Tomatis, 1990; Hemminki and Pershagen, 1994). In a recent study (Pope et al., 2002), risk factor data for approximately 500,000 adults were linked with air pollution data for metropolitan areas throughout the United States and this indicated that fine particulate and sulfur-oxide-related pollution were significantly associated with lung cancer. In that study (Pope et al., 2002) each 10-μg/m^3 elevation in fine particulates was associated with approximately an 8% increased risk of lung cancer mortality. Ambient air, particularly in densely populated urban environments, contains a variety of known human carcinogens, including organic compounds such as benzo[*a*]pyrene and benzene, inorganic compounds such as arsenic and chromium, and radionuclides (Natush, 1978), most contained within ambient particles. Coherently, several studies

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using extracts of particulate matter sampled in urban centers reported significant mutagenic effects (Tokiwa and Ohnishi, 1986; Crebelli, 1989; Green et al., 1991; Lewtas, 1993; Sato et al., 1995a, b; Farmer et al., 1996; Zhou and Ye, 1998; Batalha et al., 1999). In situ exposure protocols demonstrated a cancer promoter effect of air pollution in mice (Reymão et al., 1997; Cury et al., 2000). In addition the presence of an increased frequency of mutations in plants (Guimarães et al., 2000; Ferreira et al., 2000) and mice (Soares et al., 2003) was evidenced after chronic exposures to "real world" air pollution. These findings are supportive of the concept that low levels of air pollution are able to favor the development of tumors, diesel emissions being one of the sources of ambient pollution more probably associated with the development of malignancies. As a consequence of the aforementioned evidence, diesel particulate matter is presently considered "reasonably anticipated to be a human carcinogen" by the Report on Carcinogens of the US Department of Health and Human Services (2002).

In São Paulo the majority of public transportation is provided by approximately 17,000 buses operating on diesel. The diesel fleet in our town produces over 30% of primary particles and 25% of secondary aerosol with an aerodynamic diameter below 10 μm (CETESB, 2002). The age and technology of diesel engines in our town is quite variable and their emissions are not subjected to control. Thus, it is possible that air quality in São Paulo may benefit from a more restrictive control of diesel vehicle emissions, but the magnitude of such a benefit is not clearly known. In fact, the same scenario—large number of diesel vehicles operating without an effective control of emissions—is shared by several large urban centers of developing countries, which face the dilemma of how much money can be spent to control air pollution when other investments are also demanding.

In 2003, a bus strike paralyzed the fleet of buses in São Paulo during 3 days, from 6 to 8 of April, the complete interruption of services being achieved on the 7th. We decided to evaluate the effect of the absence of this source of pollution on the composition, mutagenicity, and toxicity of the fine particulate material collected during this period. We reasoned that the information provided by this "natural experiment" could be of use to evaluate the adverse impact of diesel emissions on urban environments, providing evidence of the possible benefits of controlling diesel emissions.

2. Materials and methods

2.1. Particle sampling and analysis

Particles were sampled on days 7 and 15 of April of 2003 (strike and nonstrike days, respectively). For this purpose we employed a high-volume sampler (Energética, Brazil) coupled with an inlet (Tisch Environmental Inc., USA) that allows the separation of particles below 2.5 μm (PM2.5) at a flow rate of 1.1 cubic meter per minute during 24 h. Our particle sampler was located on the top of the roof of our Medical School (about 15 m above the soil), which is situated on a cross road with high traffic in São Paulo, downtown. On day 7 of April, there were no buses running in this region, whereas on the 15th of April, the frequency of buses passing in the area was 913 buses/h. Because of security reasons, we could not place our sampler at ground level. Thus, particles collected at this height may not entirely reflect exposure of people at the street level but are probably adequate to mimic exposure of the millions of inhabitants of São Paulo that live and work in multifloor buildings. Particles were collected in glass fiber filters, which were dried for 24 h at 50 °C before and after particle collection for weighing. PM2.5 concentrations were 47.32 μg/m^3 on the strike day and 43.01 μg/m^3 on the nonstrike day. The selection of the 15th of April for comparative purposes was not done only on the basis of similar PM2.5 concentrations. Strike and nonstrike days were separated by only 1 week (i.e., in the same season of the year), mean temperatures were similar (19.43 and 20.95 °C, respectively), and these days were matched with regard to mean relative humidity (72.69% and 70.38%), mean wind speed and direction (1.14 and 1.01 m/s, southwest in both cases), and atmospheric pressure (694 and 693.5 mm Hg).

Half of the filters were used for toxicological studies, whereas the remaining parts were sent for other analyses, being further split for trace element and organic compound analyses. Trace element determinations of the material collected on filters were carried out by neutron activation analysis. Filter samples and elemental standards were irradiated under thermal neutron flux of the IEA-R1 nuclear research reactor for 0.5 min and 16 h. After adequate decay times, the irradiated samples and standards were measured using a hyperpure Ge detector coupled to a multichannel analyzer. Concentrations of elements were calculated by a comparative method. Blanks of filters were analyzed using the same experimental conditions adopted in the analysis of filter samples. Contribution from blank was also discounted and then the final results were expressed as a function of volume of air collected. Sulfur determination was done by X-ray fluorescence analysis, using the nonexposed part of the filters as a blank. The ratio between nonstrike/strike concentrations of hydrocarbons associated with automotive emissions (benzene, toluene, ethyl-benzene, and xylenes; BTEX) was determined after extraction with pentane, by gas chromatography/mass spectrometry. Chemical analyses were done in triplicate and the results expressed as means.

2.2. Toxicological assays

For the evaluation of toxicity, we extracted particles from the filters by ultrasonication (8 h) in distilled water. We avoided the use of organic solvents during the process of extraction since the plant bioassays employed in this study are highly susceptible to these substances. The efficiency of extraction was determined by weighing the filters before and after extraction. The weight of the filters was determined after drying them for 24 h at 50 °C. The extraction efficiencies (mass extracted/total mass) were 0.436 and 0.41 for strike and nonstrike filters, respectively. We prepared two concentrations of extracts, corresponding to 0.1 and 1 mg/L. Blank (distilled water) and controls (formaldehyde in distilled water, 1:5000 v.v.) were also prepared.

Mutagenesis of testing solutions was determined by means of the *Tradescantia* micronucleus assay (TRAD-MN) in early tetrads of *Tradescantia pallida* (Ma, 1979; Batalha et al., 1999, Guimarães et al., 2000). Briefly, young inflorescences of *T. pallida* were obtained from a plant nursery and kept in laboratory for 24 h in Hoagland's solution before treatment. After this period, cuttings were transferred to Becker jars containing the test solutions (negative and positive controls and the two concentrations of particle extracts) for 8 h. As negative control we used distilled water and the positive solution was again a formaldehyde solution, this time at a concentration of 1:10,000 in water. After treatment, the inflorescences were allowed to recover for 24 h in Hoagland's solution and then fixed in 1:3 acetic acid/ethanol solution for 24 h. Inflorescences were dissected and young anthers squashed in a solution of acetocarmine stain on a glass slide. Only preparations containing early tetrads were considered. The number of micronuclei in 300 tetrads per slide was counted at 400 × magnification, and the results were expressed as percentage (frequency of micronuclei). The counting of the micronuclei was done in coded slides, which were revealed only after the completion of the experiment.

The inhibition of mitosis of the cells of the primary meristema of the root tips of *Allium cepa* was used as an index of the toxicity of the test solutions. The induction of roots was done in onions immersed in Hoagland's solution for 5 days. After this, roots were immersed in the test solutions for 30 h and then fixed in 1:3 acetic acid/ethanol solution for 24 h. Roots were rinsed in distilled water for 10 min and then hydrolized in 1 N HCl at 60 °C for 7 min. Roots were rinsed in water again and the root tissue was dissected 1 mm from the tip, squashed in a solution of acetocarmine stain on a glass slide, and covered with a coverglass. The mitotic rate was determined by counting 300 cells at a magnification of 1000 × in coded slides and expressed as percentage.

Statistical analysis was done by computing general linear equations, using either the micronuclei frequency or the mitotic rate as dependent variable and considering terms accounting for the type and concentration of the test substance solutions and for the strike/nonstrike periods as explanatory variables. An interaction term between concentration and period of sampling was also included in the models. The level of significance was set at 5%. Statistical analysis was done with the aid of the SPSS v10.0 package.

3. Results

Table 1 presents concentrations of the trace elements obtained in the material collected on the filters, showing, as a general rule, a decrease of element concentrations on the days of strike.

The concentration of sulfur was lower on the strike day ($0.629 \pm 0.02\,\mu g/m^3$ during the strike vs. $1.424 \pm 0.08\,\mu g/m^3$ on the nonstrike day). Coherent with the results of the elemental analysis, a reduction of 39.3% of BTEX was observed on the strike day.

Table 2 presents the results of the measurements of micronuclei in early tetrads of *Tradescantia* exposed to the two concentrations of PM2.5 extracts and the data obtained for the negative and positive controls. The same results may be graphically inspected in Fig. 1. Statistical analysis indicated a significant ($P = 0.038$) reduction of micronuclei during the strike.

Table 3 and Fig. 2 present the results of the experiments focusing on the toxicity of the aqueous extracts of PM2.5, using the mitotic rate of root tips of *A. cepa* as an estimator of toxicity. Statistical analysis disclosed a nonsignificant ($P = 0.07$) effect of the strike.

Table 1
Concentrations of trace elements in PM2.5 collected on the strike and nonstrike days

Element	Strike days	Nonstrike days
As ($ng\,m^{-3}$)	3.44 ± 0.39[a]	12.91 ± 0.53
Br ($ng\,m^{-3}$)	8.91 ± 0.32	8.88 ± 0.39
Cl ($\mu g\,m^{-3}$)	3.01 ± 0.26	8.88 ± 0.39
Co ($ng\,m^{-3}$)	0.51 ± 0.04	1.14 ± 0.04
Fe ($\mu g\,m^{-3}$)	0.78 ± 0.03	1.15 ± 0.03
La ($ng\,m^{-3}$)	1.02 ± 0.47	2.33 ± 0.29
Mn ($ng\,m^{-3}$)	11.9 ± 1.8	27.5 ± 2.2
Sb ($ng\,m^{-3}$)	3.53 ± 0.06	8.73 ± 0.08
Sc ($ng\,m^{-3}$)	0.069 ± 0.010	0.141 ± 0.009
Th ($ng\,m^{-3}$)	0.144 ± 0.053	0.351 ± 0.050

[a]Contribution from blank of filter was discounted. The uncertainty of the result was evaluated using statistical counting errors of the sample and standard.

Table 2
Means and corresponding standard deviations (SD) of the percentage of micronuclei measured in early tetrads of *Tradescantia* exposed for 8 h to different concentrations of PM2.5 extracts (0.1 and 1 mg/L)

	Group	*N*	Mean	SD
Nonstrike	Blank	8	3.14	1.73
	0.1 mg/L	10	4.46	3.47
	1 mg/L	14	7.64	5.33
	Formaldehyde	12	7.43	3.99
Strike	Blank	10	3.12	1.72
	0.1 mg/L	10	3.16	1.79
	1 mg/L	23	4.27	2.78
	Formaldehyde	10	6.28	2.27

The results obtained for the negative (distilled water) and positive (formaldehyde 1:5000 v.v. in distilled water) controls are also shown. Statistical analysis indicated a significant ($P = 0.038$) reduction of micronuclei during strike.

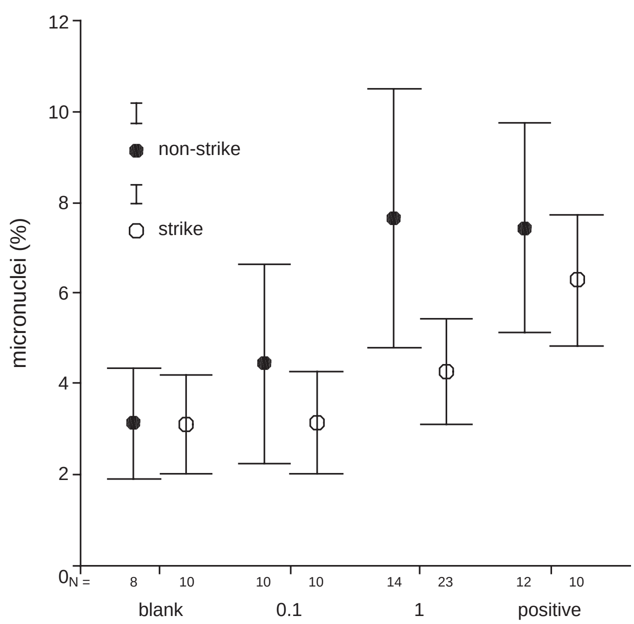

Fig. 1. Graphic representation (means and corresponding standard errors) of the percentage of micronuclei measured in early tetrads of *Tradescantia* exposed for 8 h to different concentrations of PM2.5 extracts (0.1 and 1 mg/L). The results obtained for the negative (distilled water) and positive (formaldehyde 1:5000 v.v. in distilled water) controls are also shown. Statistical analysis indicated a significant ($P = 0.038$) reduction of micronuclei during the strike.

Table 3
Means and corresponding standard deviations (SD) of the mitotic rate (%) of the meristematic tissue of the root tips of *A. cepa* exposed for 30 h to different concentrations of PM2.5 extracts (0.1 and 1 mg/L)

	N	Mean	SD
Blank	14	8.47	4.32
Formaldehyde	8	1.5	2.26
0.1 mg/L nonstrike	11	5.13	2.23
0.1 mg/L strike	7	6.81	1.95
1 mg/L nonstrike	11	4.34	3.97
1 mg/L strike	17	5.51	1.09

Results obtained for the negative (distilled water) and positive (formaldehyde 1:10,000 v.v. in distilled water) controls are also shown. Statistical analysis indicated a nonsignificant ($P = 0.07$) reduction of mitotic rate during strike.

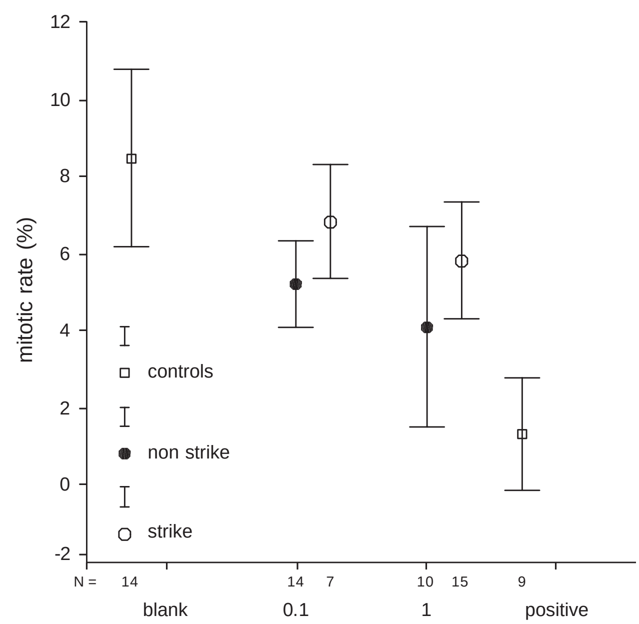

Fig. 2. Graphic representation (means and corresponding standard errors) of the mitotic rate (%) of the meristematic tissue of the root tips of *A. cepa* exposed for 30 h to different concentrations of PM2.5 extracts (0.1 and 1 mg/L). Results obtained for the negative (distilled water) and positive (formaldehyde 1:10,000 v.v. in distilled water) controls are also shown. Statistical analysis disclosed a nonsignificant ($P = 0.07$) effect of the strike.

4. Discussion

Our study showed that a bus strike in São Paulo, downtown, changed PM2.5 composition, as indicated by reductions in concentrations of some trace elements, sulfur and BTEX. When aqueous extracts of particles collected during the strike were used in assays to evaluate mutagenesis and toxicity, a significant reduction in damage to DNA was observed, without significant changes in toxicity. The finding of positive results in a plant bioassay of mutagenesis is coherent with previous reports about the mutagenic potential of urban particles employing bacterial and mammalian cell systems (Krishna et al., 1984; Whong et al., 1984; Athanasiou et al., 1986).

The observation that the mutagenic potential of ambient particles decreased when emissions generated by buses were absent is indicative that a program aiming to reduce emissions of the bus fleet in our town may impact positively the air quality by reducing the mutagenic potential of ambient particles.

Before moving further in the discussion of our results, it is important to focus first on the limitations of our study. First, we took advantage of a natural experiment, i.e., the immobilization of a large fleet of buses. This means that we could not control for the experimental conditions or even reproduce them based on any protocol. Second, we had particles sampled in one location of our town, and we cannot be sure that the changes observed in the particles sampled by us are representative of the citywide condition. Finally, the amount of PM2.5 collected during the strike period was small, limiting the possibility of conducting a large series of toxicological analyses.

The identification of how representative our sample was with respect to other parts of the town was not possible, since gravimetric measurements of PM2.5 do not exist in São Paulo, meaning that there were no filters containing PM2.5 collected from other regions of our Metropolitan Area. In addition, the length of the strike was not long enough to allow repeated measurements of changes in composition and toxicity of PM2.5 collected in the course of the absence of operating buses. We decided to evaluate particles sampled on the 7th of April, when the strike was at its maximum, with virtually no buses operating in the town.

Even with the limited amount of material available to be evaluated, we insisted on having measurements of our sample including estimators of both its composition and its biological effects. We reasoned that by having data on these two perspectives, the strength of our results would increase, since both types of data provide complementary pieces of information.

The changes in particle composition are observed even when compared with a day with an almost equivalent mass concentration and with similar metereological conditions. Our results indicate that, for the same mass of PM2.5, changes in particle composition may alter its deleterious effects. This is an important topic and we think that our results reinforce the concept that particle speciation is necessary when dealing with health effects and control policies. Viewed only from the perspective of mass, one could conclude that control of the emissions of buses do not influence air pollution by fine particles. Indeed, PM2.5 levels on the day of the strike were high, above 40 $\mu g/m^3$. This high level can be explained by the intense traffic jam that our town experienced with the lack of operating buses. São Paulo has a program that takes out of circulation 20% of the automotive fleet based on a rotation determined by the last digit of the vehicle license (numbers 1 and 2 do not run on Mondays, 3 and 4 on Tuesdays, etc.). During the strike, all vehicles were allowed to circulate to compensate for the lack of public transportation. In addition, light duty diesel vehicles, such as vans, were also liberated to operate in São Paulo, helping people to get to their jobs. In fact, we are reporting the results of an event that stopped heavy-duty vehicles but increased the traffic of gasoline, ethanol, and light-duty diesel-fueled mobile sources.

Considering the small amount of material available, we decided to employ short-term higher plant bioassays to evaluate particle mutagenicity and toxicity. These assays allow one to evaluate a large number of individuals using low volumes of the test sample, and these assays possess high sensitivity. In fact, plant bioassays are ideally suited to perform short-term studies of mutagenesis, both in laboratory and in situ biomonitoring investigations (Ma, 1979, 1981; Ichikawa, 1981; Watts et al., 1989; Rodrigues et al., 1997; Batalha et al., 1999; Ferreira et al., 2000; Guimarães et al., 2000).

Among the mutagenesis tests based on plants, the TRAD-MN is considered perhaps the most sensitive (Ma, 1981; Watts et al., 1989). Briefly, the test is based on the visualization of segments of chromosomes as small round and dark structures (micronuclei) in early tetrads of pollen mother cells. The micronuclei represent the consequence of defects that occurred during chromosome meiotic replication of the pollen progenitor cells. After exposure to mutagenic agents, the frequency of micronuclei formation increases, allowing the determination of the degree of chromosomal damage during meiosis. Our *Tradescantia* system worked quite well, disclosing similar values of baseline frequency of micronuclei on two separate occasions, expressed similar increased values of micronuclei in very low doses of formaldehyde (Fig. 1), and exhibited a dose–response increase of micronuclei when exposed to PM2.5 collected on the nonstrike day.

We considered the inhibition of mitosis in root tips of *A. cepa* an estimator of cellular toxicity. Mitosis is very sensitive to the external environment and mitotic arrest is an event shared by dividing tissues when subjected to injury. The study of root tip dividing cells is considered a sensitive indicator of toxicity (Cotelle et al., 1999). Indeed, our system exhibited a marked reduction of mitotic rate for very low concentrations of formaldehyde, our positive control, and disclosed a dose–response reduction when facing increasing concentrations of PM2.5 extracts (Fig. 2).

Based on our results, the stopping of buses did not influence PM2.5 toxicity (or have a marginal effect) (Fig. 2) but changed its mutagenic potential (Fig. 1). We did not aim to explore the capacity of urban particles to contain substances that cause damage to DNA, since this observation is not new. However, we tested the hypothesis that a single source—the bus fleet of São

Paulo—influences particle mutagenic potential, in real world conditions.

We aimed not to demonstrate that diesel particles contain mutagenic substances but to determine whether the interruption of a single source has the potential to modify their mutagenic potential, in "real world" conditions. There are presently several technological options that may reduce dramatically the emissions of particles by heavy-duty diesel engines, for example, the use of other fuels such as ethanol or biodiesel, and the use of cleaner diesel and engine control technology may reduce particle emissions up to 200 times (McClellan, 1986). Because the aforementioned measures are costly, it is worth determining in advance the benefit of implementing strategies to achieve cleaner diesel emissions. We think that our study provides a solid argument in favor of directing policies to control the emissions of diesel buses. In a town that houses millions of mobile sources, the control of emissions of a few thousand of them seems to be a cost-effective procedure in a scenario of limited resources to invest in air pollution control.

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