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RESEARCH ARTICLE

Monitoring human genotoxicity risk associated to urban and industrial Buenos Aires air pollution exposure

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Abstract

The quality of life in large megacities is directly affected by its air quality. In urban environments, suspended particles from anthropogenic origin is one of the main air contaminants identified as highly genotoxic, mutagenic, or carcinogenic. Atmospheric monitoring is therefore imperative, and bioassays to detect the effects of genotoxic agents give usually excellent results. Analysis of micronucleus (MN) in exfoliated oral mucosa cells is a sensitive non-invasive method for monitoring genetic damage in human populations. The first aim of this study was to analyze and characterize levels of volatile organic compounds (VOCs), particulate matter (PM), and polycyclic aromatic hydrocarbons (PAHs) in two areas from Buenos Aires: La Plata city, an urban (U) area and Ensenada, an industrial (I) area. Secondly, we evaluated the possible health risk of its inhabitants through a simple genotoxic assay on exfoliated oral mucosa cells. Whole blood cell count and nuclear abnormalities frequencies were evaluated in the exfoliated oral mucosa cells from urban and industrial inhabitants. Smoking habit represented a significant factor increasing MN percentage while, age did not increase the production of any of the nuclear aberrations assayed (micronuclei, binucleated, karyorrhexis) when the inhabitants from the urban and the industrial areas were compared. In addition, changes in MN and binucleated cell percentages in males and females were found to be area-dependent. We suggest that regardless PM concentration, PM-specific characteristics (size, shape, chemical elements, etc.) and VOCs levels could be responsible for the different harmful genotoxic effects seen in the two areas. Although this is a preliminary study, our results allowed to recognize that individuals living in both the urban and the industrial areas could be considered susceptible groups and should periodically undergo biological monitoring and appropriate care.

Keywords Air pollution · Urban environment · Industrial environment · Genotoxicity · Micronucleus

Introduction

Air pollution consisting of gases and particulate matter (PM) represents a health problem in urban centers worldwide resulting in 6.5 million premature deaths annually with 90% mortality in low or median-income countries (WHO 2018). The 54% of the world population live in urban areas where industrial and urban exhausts are the two main sources of air pollution. However, environmental air pollution depends not only on the source of emission and the quantities emitted but also, on local factors such as topography and weather conditions, therefore each city is a unique system.

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In general, in urban areas, the number of vehicles is increasing three times faster than the rate of population growth, being diesel-burning buses and trucks the major source of airborne contamination. La Plata is the capital city of Buenos Aires Province, Argentina that according to the National census (INDEC 2011) has a population of 799,523 inhabitants. Public transportation network in La Plata city is very dense; vehicular traffic is intense comprising around 350,000 cars, 3100 taxis and rent-cars, and 640 buses (Frediani and López 2014). The vehicle fleet in this city is one of the principal sources of air pollution which contribution has increased significantly in the last 20 years (Colman Lerner et al. 2014b). Because of its demographic and transportation profile and because this city is placed in an area from Buenos Aires where the atmosphere has low self-cleansing capacity, vehicle combustion exhaust should be considered as an important cause of deterioration of La Plata atmosphere.

In the developing world, it is not uncommon to find in certain locations, a concentration of heavy industries, designated by governments as "development poles." One of the most important refineries in South America and one of the most dynamic industrial pole in Argentina is placed in the city of Ensenada. The refinery has a refining capacity of 200,000 barrels of crude oil per day obtaining a wide range of products with a yearly production of more than 650,000 tons. This refinery includes a production facility of base lubricants, paraffin, aromatics, asphalt, and petrochemical products. In this area, the rapid introduction of new and complex processes into a social context, not well prepared to control their potential associated risks (dumping of toxic wastes and by-products of technological processes), can result in serious consequences to the environment and the health of its inhabitants. Moreover, fine particles suspended in the air and PAHs associates, resulted from the incomplete burning of biomass and fossil fuels, leave a deposit of soot on surfaces, and have proven to be a major lung irritant (E. Ratto et al. 2018). Furthermore, Ensenada with 56,129 inhabitants (INDEC 2011) is not only an industrial, but a port which in 2014 was expanded and modernized having an operational capacity of 400 thousand containers per year.

Populations from urban and industrial areas are constantly exposed to an array of airborne toxic agents that might cause genetic damage, congenital defects, carcinogenesis, and shortened life expectancy (Hirvonen 1995; Brook et al. 2002; Kok et al. 2009; Sosa et al. 2017; Colman Lerner et al. 2018). In particular, La Plata conglomerate (La Plata city and its surrounding) has been considered one of the six most potentially air-polluted areas of Argentina (Petcheneshsky 1996) being air pollution one of the most important anthropic risks. In this sense, identifying and evaluating these hazardous xenobiotics might help take actions to decrease their presence in the environment and to minimize their potential adverse consequences on people's health.

In this regard, the first step to study the effects on exposed populations, is to conduct monitoring studies using pertinent equipment to analyze the air pollution. Secondly, it is important to screen the population by means of simple methods in order to detect and identify possible early-stage DNA damage.

Micronucleus (MN) assay in oral mucosal cells is not only a good indicator of chromosome mutations but a non-invasive, simple, rapid, and cheap test applicable to many cell types (Majer et al. 2001), frequently used in earlier occupational studies (Wultsch et al. 2014; León-Mejía et al. 2014; Idolo et al. 2018; Villarini et al. 2018). This assay allows not only to detect MN but also disturbances of the mitotic cycle leading to the formation of binucleated cells and other anomalies reflecting cytotoxic effects (karyorrhexis).

Micronuclei originate from structural and numerical chromosomal aberrations (Norppa and Falck 2003) and consist of acentric chromosomes, chromatid fragments, or whole chromosomes that have failed to be incorporated into the daughter nuclei during mitosis. The MN index in rodent and/or human cells has become one of the standard cytogenetic endpoints and biomarkers used in genetic toxicology in vivo or ex vivo. MN assay has been employed for exposed urban populations worldwide as an "endogenous dosimeter" in tissues that are specific targets of genotoxic and carcinogenic agents including particulate matter (Roubicek et al. 2007; Sellappa et al. 2010; Mørck et al. 2016). In humans, MN can be easily assessed in erythrocytes, lymphocytes, and exfoliated epithelial cells to obtain a measure of genome damage induced in vivo (Feretti et al. 2014; Bonetta et al. 2019). Further, the increased frequencies of MN in the lymphocytes have been associated with cancer risks (Bonassi et al. 2011).

The oral mucosa cells constitute a relevant tool in the biomonitoring of populations exposed to genotoxic and mutagenic agents (Holland et al. 2008). It has to be pointed out that MN in exfoliated cells is induced when the cells are in the basal layer, thus reflecting the genotoxic effect occurred during the last 3 weeks. In addition to MN, meta-nuclear alterations such as binucleated cells, karyorrehxis, pyknosis, and chromosome bridges must be taken into account, as they are also indicative of genotoxicity (Tolbert et al. 1992).

The aim of this study was first to analyze and characterize air particulate matter of two different areas from Buenos Aires: La Plata city, an important vehicular transit area and Ensenada city, an industrial pole; and secondly to evaluate the possible health risk of its inhabitants through a simple genotoxic assay on exfoliated oral mucosa cells.

Materials and methods

Urban and industrial areas

The coastal region of the edge of the Río de la Plata located in the north-eastern area of the province of Buenos Aires is an important socioeconomic development. La Plata, Berisso, and Ensenada cities particularly represent an area with strong anthropogenic activity (Kruse et al. 2011; Colman Lerner et al. 2014b). The coastal region is humid, owing to its coastal location; its average monthly humidity is higher than 65% and the average annual temperature is around 18–16 °C. As for the wind, its average annual intensity reaches 12–18 km/h, with prevailing winds coming from the East, Northeast, and Southwest (Fig. 1a).

La Plata (34° 55′ 17.2″ S, 57° 57′ 16.3″ W) with a population of 799,523 inhabitants (INDEC 2011) and a dense public transportation network is the capital city of Buenos Aires Province, Argentina. Placed in a Buenos Aires area where the atmosphere has low self-cleansing capacity, vehicle combustion exhaust should be considered the main cause of its atmosphere deterioration.

The party of Ensenada is located to the East of the Province of Buenos Aires, on the South coast of the River of La Plata. It is 7 km from the city of La Plata and 65 km from the Autonomous City of Buenos Aires (Fig. 1). The City of Ensenada (34° 86′ 48″ S, 57° 90′ 5″ W) located by the small Ensenada Bay on the Río de la Plata has a population of 56,729 inhabitants (INDEC 2011). The port activity and the Free Zone and the Petrochemical Complex, rated as one of the most important South America, highlight its industrial, productive, and developmental nature of this city.

In this context, and due to the characteristics of the cities mentioned above, we considered La Plata as an urban area (U) and Ensenada as an industrial area (I).

Particulate matter collection and morphochemical characteristics

Particulate matter collection

Particulate matter levels both for PM_{10} and $PM_{2.5}$ were monitored using MiniVol TAS (AirMetrics Co., Springfield, Oregon, USA) that draw air at 5 L/min sequentially through a particle-size separator (impactor) and filter thereby capturing PM; polytetrafluoroethylene (PTFE) membrane of 46.2-mm diameter and 2-μm pore-size was used as the filter. Both particles of size < 2.5 μm ($PM_{2.5}$) and aerodynamic diameter ≤ 10 μm (PM_{10}) were detected. Gravimetry was used to determine the particle content in each sample. PM collection was carried out simultaneously in both areas following the methodology of (Gutierrez et al. 2019).

PM morphological and chemical composition study

Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were employed to analyze particle morphology, size, and chemical composition from the industrial and the urban areas. Samples were analyzed

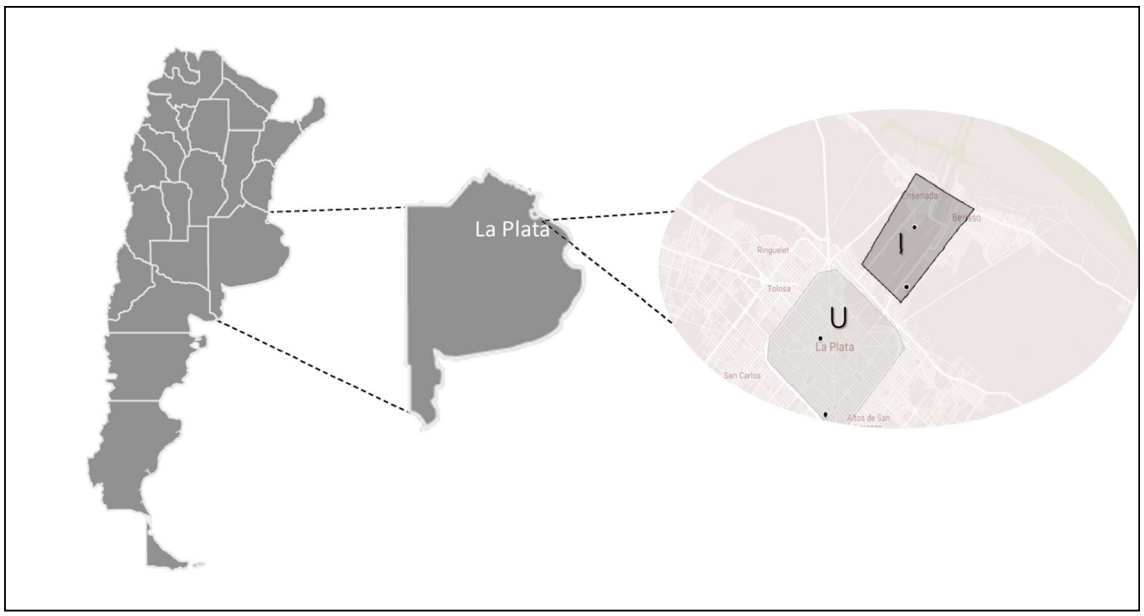

Fig. 1 Map of Argentine and Buenos Aires Province. Study areas are circumscribed: industrial zone (dark gray box) and urban zone (light gray box). Monitoring points in each area are shown as black dots

using a Philips SEM 505 with EDS PRIME 10 Microprobe System.

Polycyclic aromatic hydrocarbons (PAHs)

The presence of PAHs was analyzed using high-performance liquid chromatography (HPLC) with fluorescence detection. 16 US EPA priority PAHs were detected: naphthalene (Naph), acenaphthylene (Acpy), acenapthene (Acp), fluorene (Flu), phenantrene(Pha), anthracene(Ant), fluoranthene(Fl), pyrene(Pyr), benzo(a)antrhacene(BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene(BkF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene(Ind), dibenzo(a,h)anthracene(DBA), and benzo(g,h,i)perylene(BghiP). The methodology was performance like (Sosa et al. 2017).

Volatile organic compounds (VOCs)

The methodology used consisted in performing an active monitoring of VOCs (coal tube, skc17-01A) in the two study areas, at a flow rate of 0.2 l min-1 during 8 h. The samples were analyzed by gas chromatography with mass spectrometry detector. The detection limits for all components were estimated as three times the standard deviation (SD) of five repeated measurements of unused (blank) tubes. Values of 0.01 to 0.05 ng m^{-3} were obtained for 8 h of sampling.

We analyzed 24 VOCs: hexane, ethyl acetate, chloroform, cyclohexane, carbon tetrachloride, benzene, 1,2-dichloroethane, heptane, 3-methyl-2-butanone, n-propyl acetate, methyl isobutyl ketone, toluene, perchlorethylene, acetate of butilo, chlorobenzene, ethylbenzene, m + p-xilene, nonane, o-xilene, ethoxyethyl acetate, cumene, cyclohexanone, undecane, and dodecane.

Study population

The study involved 57 volunteers (n) whose ages range between 22 and 67 years old, living and working in: La Plata ($n = 31$) and Ensenada ($n = 26$), two different locations from Buenos Aires.

To eliminate different confounding factors that can influence chromosome mutation percentage, individuals from the industrial and the urban areas were chosen in a way so that they were comparable in age distribution, ethnicity, food lifestyle, nutritional status, as well as for the extent of indoor air pollution at their homes. Exclusion criteria included pregnancy or breastfeeding (in female volunteers), drug addictions, and smoking. The main characteristics showing the profile of the subjects who took part in this study are presented in Table 1.

All individuals signed a post-informed consent before inclusion in the study. Note: at the time of the study, none of the participants was under medical treatment involving drugs of any kind. The study was conducted according to the tenets of the Declaration of Helsinki(World Medical Association. 2001). The research protocol was approved by the Central Advisory Committee on Bioethics (*Comité Consultivo Central de Bioética*) of UNLP, and informed consent was obtained in all subjects before they were registered in the study.

Blood samples and analysis

Venous blood samples (10 mL) were collected from each individual by standard procedures and whole blood cell (WBC) count, and evaluation of its subpopulations was performed using an automatic biochemistry analyzer (Metrolab CM 250, Weiner lab). To prevent blood from clotting, 1.5 mg/mL ethylene diamine tetra acetic acid (EDTA) was added in each tube. Patients from both the industrial and the urban area fasted 12 h previous to the blood extraction.

Sample collection and nuclear aberrations assay

Exfoliated epithelial cells from oral mucosa were collected by gently scraping the oral mucosa from the middle part of the inner cheeks with a wooden spatula. The cells were washed three times in 0.9% phosphate saline, then cells were smeared on slides, dried in air, and fixed with cold solution of 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2–7.4) for 20 min. Slides were stained with Giemsa following Fenech et al. (2003) as was the criteria for the scoring of MN. Briefly, MN were scored when MN diameter was 1/3 of the main nucleus diameter or less, when they were round shaped, not refractile, not connected to the main nucleus, when they touched but not over-lapped the main nucleus, and when their chromatin structure and staining color were similar to those of the main nucleus.

Besides MN, other nuclear anomalies pyknosis (shrunken nuclei), karyorrhexis (nuclear disintegration), karyolysis (dissolution of nucleus), and binucleated cells were evaluated following the criteria of Tolbert et al. (1992). No less than 2000 cells from each person were analyzed and scored under light microscope with × 40 and × 100. Results are expressed as percentage of cell nuclear aberration.

Statistical analysis

The data acquired were tested for normality and heterogeneity of variance using Chi-Square analysis and Bartlett's test, respectively. Analysis of nuclear abnormalities was performed using a non-parametric Mann-Whitney test. Data are presented as the mean ± SD. p value of < 0.05 was considered to indicate statistical significance. All calculations were

Table 1: Demographic population characteristics from Ensenada and La Plata zones.

Parameters	Industrial zone Ensenada		Urban zone La Plata	
	Males ($n = 16$)	Females ($n = 10$)	Males ($n = 11$)	Females ($n = 20$)
Range eye (years)	28-59	37-60	24-56	22-67
Current smoker (%)	56.3	30	9.1	10
Food habit (%)	b=87.5; f= 12.5	b=90; f=10	b=100	b=85; f=10 v=5
Weight (kg)	85.16 ± 14.02	74.56 ± 13.75	80.64 ± 13.43	60.70 ± 11.84
Height (m)	1.73 ± 0.05	1.62 ± 0.06	1.76 ± 0.09	1.64 ± 0.06
BMI (kg/m2)	28.49 ± 3.86	28.58 ± 5.20	25.94 ± 3.93	22.54 ± 3.15

F= female, M=male, b=balanced, v=vegetarian, f=rich in fats

performed with Infostat software (Universidad Nacional de Córdoba, Córdoba, Argentina) and Graphpad Prism.

Results

Particulate matter collection and morphochemical characterization

PM_{10} and $PM_{2.5}$ concentration from industrial and urban areas are shown in Table 2. PM_{10} level was significantly higher in the industrial zone when compared with the urban area ($p < 0.05$) while $PM_{2.5}$ level showed not statistically significant differences between areas.

The analysis of the micrographs is shown in each area (Fig. 2); the greatest variety of morphologies are found in the samples of the industrial area, standing out besides irregular particles, particle conglomerates, and particles of spherical shape (PM_{10}). Spherical particle morphology (mostly free or in conglomerates) is associated mainly to high temperatures incomplete combustion as occurs in the petrochemical pole located in the industrial region (Breed et al. 2002; Li and Shao 2009; González et al. 2017). Irregular geometry prevails in particle conglomerates. On the other hand, in the urban area, the lack of spherical particles is evident, with irregular geometry particles prevailing over the particle conglomerates.

The elemental analysis by EDS for both PM_{10} and $PM_{2.5}$ shows a marked difference in the carbon (C) level (~ 2 fold) when the two areas under study were compared. As was previously reported by González et al. (2018) and Dappe et al. (2018), the Ensenada area showed high content of C and low content of metal traces indicative of industrial activity being the main emission sources of the petrochemical industry and the production of petroleum coal (Fig. 3).

PAHs

PAHs concentration from industrial and urban areas are shown in Table 2. Although PAHs levels were not significantly different in both areas, BaA which have been identified as a compound associated to cancer risk included in group 1 by IARC was detected.

VOCs

Table 2 shows VOCs concentration detected in both areas studied. Form the 24 VOCs analyzed, 13 species presented values higher in the industrial with respect to the urban area being this differences statistically significant ($p < 0.05$). Among them, according to IARC, 1,2 dichloroethane and benzene are considered possibly carcinogenic where benzene was identified as carcinogenic to humans. In our populations, although the average industrial values were higher, if we take into account the LCR index (Life time cancer risk), following the methodology of Colman et al. (2013 and 2018), both populations have values of 4.2×10^{-7} (I) and 1.5×10^{-7} (U). The WHO considers as acceptable a LCR between 1×10^{-5} and 1×10^{-6}, whereas values lower than 10×10^{-6} were recommended by USEPA.

Sample collection and nuclear aberrations assay

In order to validate our experimental protocol, we first analyzed micronuclei (MN) presence in the smoker and non-smoker populations from the industrial area (Fig. 4). MN percentage showed a significant difference between both populations assayed (non-smoker = 0.74 ± 0.52 vs. smoker = 2.35 ± 1.08 mean ± SD, $p < 0.05$). This result shows that smoking is a factor that significantly modifies MN percentage.

Therefore, as smoking was found to be a cofounding factor which can mask air pollution effects on oral mucosa cells, we followed our study only by analyzing the non-smoker population from both the industrial and the urban areas. The comparative analysis, only for the non-smoker population, showed no differences in the MN percentage between areas (Fig. 5). Nevertheless, as nuclear aberrations could vary with sex, we sought to determine the MN, binucleated cell, and karyorrhexis percentage for non-smokers females vs. males within the two areas (Fig. 6).

Furthermore, we also analyzed MN, binuclated cells, and kariorrhexis frequency with regard to age. We

Table 2: PM, VOCs and PAHs concentrations from Ensenada and La Plata zones.

		Industrial zone Ensenada		Urban zone La Plata	
		n	mean ± SD	*n*	mean ± SD
PM	PM_{10} [μg/m^3]	14	52.2 ± 16.7*	14	31.9 ± 13.9*
	$PM_{2.5}$ [μg/m^3]	26	16.8 ± 9.5	15	16.7 ± 12.2
VOCs	Hexane	22	7.7 ± 7.4*	25	2.5 ± 5.0*
	Ethyl acetate	22	3.3 ± 4.3	25	2.1 ± 3.1
	Chloroform	22	0.1 ± 0.4	25	0.1 ± 0.4
	Cyclohexane	22	2.97 ± 3.33	25	1.96 ± 3.95
	Carbon tetrachloride	22	0.2 ± 0.68	25	0.09 ± 0.33
	Benzene	22	0.56 ± 0.72*	25	0.2 ± 0.19*
	1,2-dichloroethane	22	0.16 ± 0.64*	25	0.00029 ± 0.0015*
	Heptane	22	0.46 ± 0.74	25	0.2 ± 0.2
	3-methyl-2-butanone	22	0.06 ± 0.27	25	0.03 ± 0.15
	n-propyl acetate	22	0.0027 ± 0.01	25	0.00032 ± 0.0016
	Methyl isobutyl ketone	22	0.15 ± 0.17*	25	0.05 ± 0.06*
	Toluene	22	3.3 ± 3.4	25	1.72 ± 1.86
	Perchlorethylene	22	0.81 ± 1.49	25	0.46 ± 0.66
	acetate of butilo	22	2.01 ± 6.45*	25	0.41 ± 0.34*
	Chlorobenzene	22	0.02 ± 0.01*	25	0.01 ± 0.01*
	Ethylbenzene	22	0.38 ± 0.67*	25	0.13 ± 0.09*
	m+p-Xilene	22	2.73 ± 4.01*	25	0.95 ± 0.76*
	Nonane	22	2.59 ± 4.96*	25	0.64 ± 0.58*
	o-Xilene	22	3.93 ± 6.09*	25	1.05 ± 1.13*
	Ethoxyethylacetate	22	2.55 ± 7.95	25	ND
	Cumene	22	0.33 ± 0.63	25	0.14 ± 0.15
	Cyclohexanone	22	8.37 ± 17.97*	25	2.26 ± 1.43*
	Undecane	22	7.63 ± 7.76*	25	3.36 ± 3.35*
	Dodecane	22	7.33 ± 6.5*	25	3.29 ± 2.72*
HAPs	Naph	15	ND	8	ND
	Acpy	15	ND	8	ND
	Acp	15	ND	8	ND
	Flu	15	4.14 ± 8.36	8	6.38 ± 11.87
	Pha	15	1.17 ± 2.81	8	1.11 ± 2.5
	Ant	15	7.63 ± 12.46	8	7.15 ± 10.2
	Fl	15	0.02 ± 0.04	8	ND
	Pyr	15	ND	8	ND
	BaA	15	0.14 ± 0.16	8	0.1 ± 0.28
	Chr	15	ND	8	ND
	BbF	15	0.42 ± 0.47	8	0.08 ± 0.08
	BkF	15	0.27 ± 0.31	8	0.19 ± 0.37
	BaP	15	0.13 ± 0.11	8	0.1 ± 0.27
	DBA	15	0.03 ± 0.07	8	ND
	BghiP	15	0.58 ± 0.45	8	0.25 ± 0.44
	Ind	15	3.33 ± 10.07	8	0.87 ± 2.47

Results are expressed as the mean ± SD. Asterisks (*) show statistical significance at p <0,05. ND= Not detectable

evaluated nuclear aberrations within three age intervals: young adult (18–35), middle-aged adult (36–55), and older adult (> 55) as described elsewhere (Petry 2002). No differences were detected for any of the nuclear aberrations among the different age groups evaluated (data not shown).

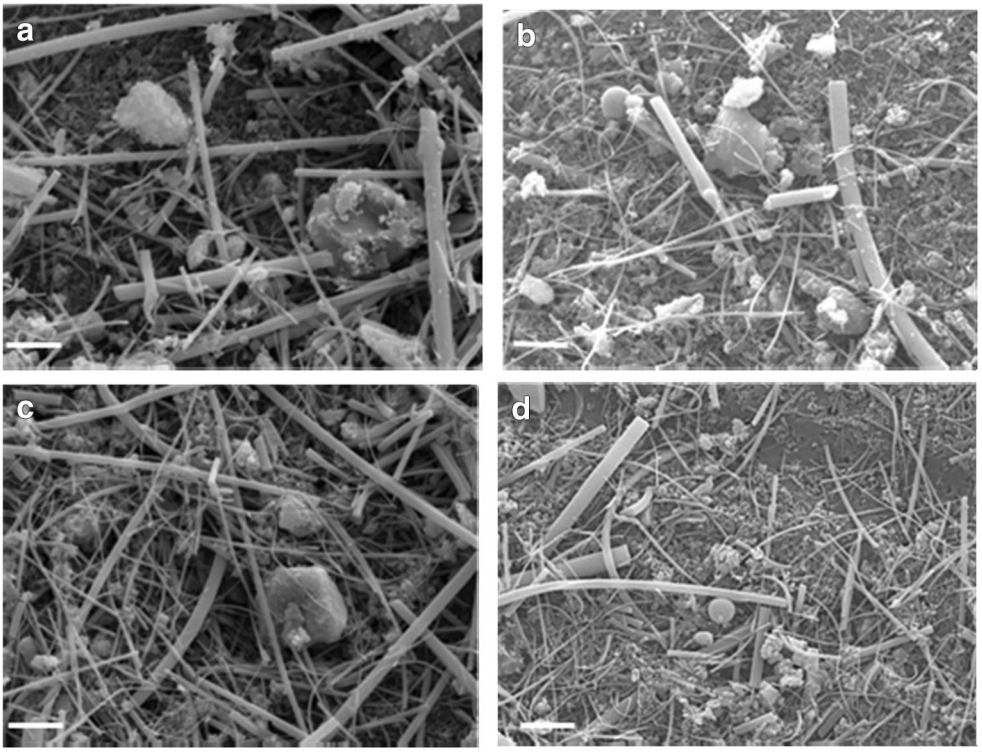

Fig. 2 Particulate matter morphology. Representative SEM images of samples collected in urban and industrial zones: **a** U PM_{10}, **b** I PM_{10}, **c** U $PM_{2.5}$, and **d** I $PM_{2.5}$

Blood biochemical analysis

Physiologic and pathologic processes may cause certain alterations in the total and absolute differential whole blood cell counts. Therefore, the identification of the leukogram pattern is key to the interpretation of changes in hemogram results. Toxic changes are frequently seen as part of an inflammatory leukogram. As smoking was found to be a strong cofounder influencing MN percentage, the biochemical study was carried out only by analyzing the non-smoker individuals from both the industrial and the urban areas. We found no changes in the leukogram pattern neither in the population exposed to

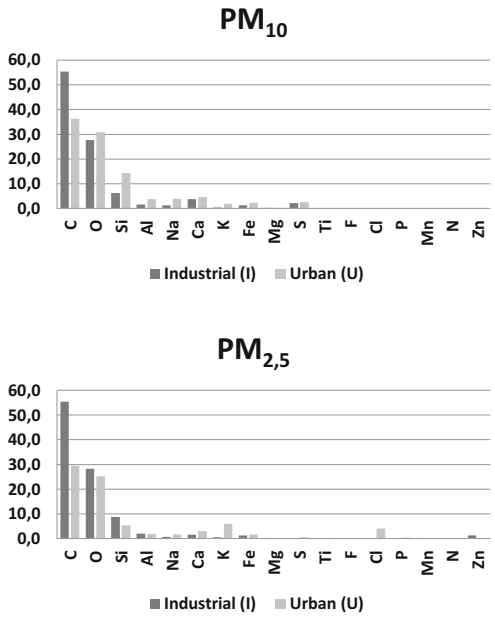

Fig. 3 Particulate matter chemical composition from industrial and urban zones. Elemental analysis was performed by SEM-EDS

	PM_{10}		$PM_{2.5}$	
	I	U	I	U
C	55,4	36,2	55,4	29,5
O	27,7	30,9	28,2	25,2
Si	6,2	14,4	8,7	5,4
Al	1,6	3,8	2,0	1,8
Na	1,2	3,8	0,7	1,7
Ca	3,8	4,7	1,5	3,1
K	0,4	1,8	0,5	5,9
Fe	1,3	2,3	1,3	1,7
Mg	0,2	0,3	0,3	0,1
S	2,2	2,5	0,0	0,5
Ti	0,0	0,0	0,0	0,2
F	0,0	0,1	0,0	0,0
Cl	0,1	0,0	0,0	4,0
P	0,0	0,0	0,0	0,4
Mn	0,0	0,0	0,2	0,0
N	0,0	0,0	0,0	0,0
Zn	0,0	0,0	1,2	0,0

Fig. 4 Box and whisker plots of micronuclei from smokers and non-smokers in the industrial zone. Median (solid line), mean (dotted line). Asterisks (*) show statistical significance at $p < 0.05$

industrial air pollution nor in the population exposed to urban air pollution (Table 3).

Statistical analyses

The data acquired were tested for normality and heterogeneity of variance using Chi-Square analysis and Bartlett's test, respectively. Student's *t* test was performed for normally distributed data, to determine statistically significant differences between two means. *p* value of < 0.05 was considered to indicate statistical significance. All calculations were performed with Infostat software (Universidad Nacional de Córdoba, Córdoba, Argentina) and the box graphics with the Graphpad Prism software.

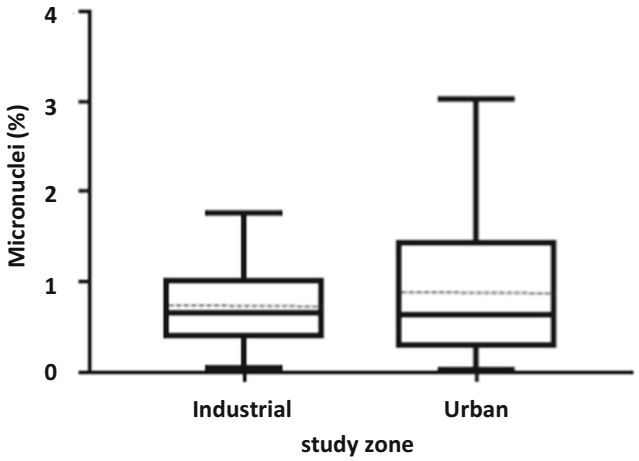

Fig. 5 Box and whisker plots of micronuclei from non-smokers in the industrial and urban zone. Industrial zone: Ensenada; Urban zone: La Plata median (solid line), mean (dotted line). No significant differences were found between zones

Discussion

Megacities atmospheres contain complex mixtures of air pollutants including mutagenic and carcinogenic substances such as diesel soot, heavy metals, and semi volatile organic compounds (VOCs) such as polycyclic aromatic hydrocarbons (PAHs). Oral mucosa cells are the first barrier for the inhalation route for air pollutants and are capable of metabolizing proximate carcinogens to reactive products (Vondracek et al. 2001; Spivack et al. 2004). Approximately 90% of human cancers originate from epithelial cells (Rosin 1992). Thus, it could be argued that oral epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body mainly via inhalation and ingestion. In this context, herein we studied the exfoliated oral mucosa cells profile and its possible association to environmental PM levels from two different areas of Buenos Aires megacity: the city of Ensenada, an industrial area (I) and the city of La Plata, an urban area (U).

The industrial area is mainly related to the national petrochemical industry and the generation of petroleum coke. Both industries are important sources of PM emission, with the petrochemical industry also being an important source of VOC's emissions. Previous studies showed that VOC's levels in this area are higher than in the urban area. More recently, as traffic became an important source of emission in both regions, VOC's levels have substantially increased (Massolo et al. 2009; Colman Lerner et al. 2014a, b).

Regarding PM concentration, annual averages for both sampling sites exceed guideline values of 20 $\mu g/m^3$ (WHO 2006). It is noteworthy to point out that in the industrial area, PM levels (mainly PM10) almost doubled the amount of PM at the urban site (Wichmann et al. 2009; Colman Lerner 2013) exceeding not only the WHO guidance values but also the local legislation guideline values (Vidal 2018). Air particles from the industrial and urban areas analyzed by SEM-EDS showed distinct morphology as well as differences in carbon levels, which were always higher in the industrial zone. The latter might be probably associated with emissions from the petrochemical pole. Even though no differences in PAHs concentration were observed between the areas studied, the presence of BaA identified by IARC as carcinogenic was detected. Average VOCs levels were always higher in the industrial area including carcinogenic (benzene) or possibly carcinogenic (dichloroethane and benzene) species, although based on the LCR (Life time Cancer Risk), both populations in accordance with WHO and EPA, have acceptable values. These results could reflect the petrochemical pole activity (Colman Lerner et al. 2013, 2014a, b). As shown herein, PM morphological

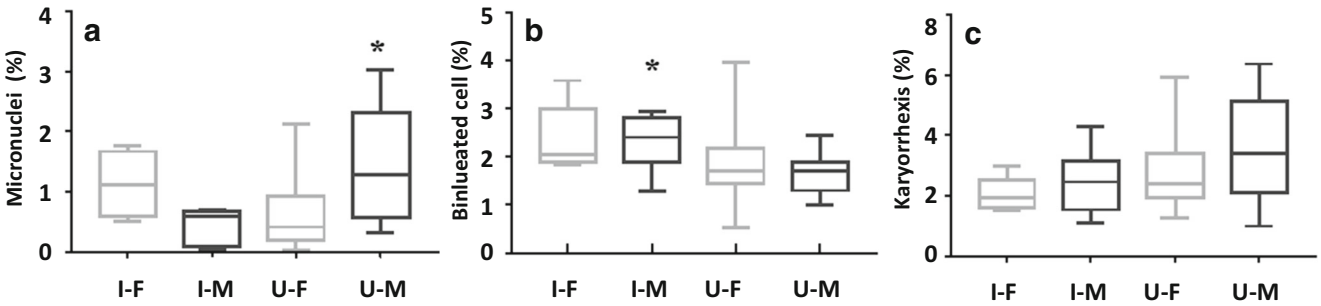

Fig. 6 Nuclear aberration by zone and gender. **a** Micronuclei. **b** Binucleated cell. **c** Karyorrhexis cell percentage. I, industrial zone (Ensenada); U, urban zone (La Plata); F, female; M, male. Box and whisker plots. Asterisks (*) show statistical significance at $p < 0.05$. Median (solid line)

and chemical determination allows us identify air quality differences in the two studied areas.

To detect and identify possible early-stage DNA damage in the individuals exposed to different PM levels, we monitored employing a simple method, the frequency of nuclear aberrations in exfoliated oral mucosa cells from subjects living and working in two areas (U and I) from Buenos Aires.

The micronuclei (MN) frequency variation observed within the exposed groups, characterized by the high values of the standard deviation, must be due to the fact that the response to a given genotoxic agent is different from person to person. This differential response may be the result of different factors, such as genetic constitution and life habits. In this sense, sex and the habit of smoking are widely considered confounding factors (Maffei et al. 2002). Therefore, to establish whether these factors could exert any additional effect, we determined the frequency of micronuclei between smokers vs. non-smokers. The smoking habit is widely accepted as a major cause of bladder cancer, since 50% of the cases were identified in smoking men and 33% in smoking women (Silverman et al. 1992). When analyzing exfoliated oral mucosa cells Fontham et al. (1986), detected a sixfold increase in the number of MN in smokers; Reali et al. (1987) also reported an increase in the number of MN, and Burgaz et al. (1995) found a significant increase in micronucleated cells ($p < 0.001$) in smokers, as compared to non-smokers. In accordance with these and other researchers, we found a highly significant increase in the micronuclei frequency ($p < 0.001$) between smokers and non-smokers from the industrial area.

Although many studies report the age and gender of the subjects studied, only a fraction were able to establish a statistically significant effect by age (Gattás et al. 2001; Özkul et al. 1997) or by gender (Fenech et al. 1999; Pastor et al. 2001). When MN frequency in relation to the age of the individuals was evaluated, no differences are found between the two populations assayed (data not shown).

On the other hand, when MN frequency within each populations was evaluated with regard to sex, we observed significant differences between male–female MN. The same pattern was observed for binucleated cells. These results could be reflecting the greater chromosomal instability that men present with respect to women, making them more sensitive to genotoxic effects.

Because of the significant increase of modified chromosomes which happens together with the increase in age, irrespective of the gender, some studies show a positive association between age and MN frequency (Nath et al. 1995; Calvert et al. 1998; Fenech et al. 1999).

Frequency of micronucleated cells was 7.84 ± 7.35 per 1000 in the industrial population and was shown to have a large inter-individual variability. The study of factors contributing to this variability showed that smoking and sex could affect nuclear aberration rate.

The results allowed to conclude that the individuals living both in the industrial and urban areas, where the air quality are

Table 3. Industrial and urban population leukogram pattern.

	Industrial zone Ensenada		Urban zone La Plata		Reference valor
	mean ± SD	range	mean ± SD	range	range
Total Leukocytes	7.2 ± 2.2	2.9 – 9.9	6.6 ± 2.1	2.9 – 11.9	5.0 - 10
Lymphocytes	2.4 ± 0.6	1.5 – 3.2	2.4 ± 0.7	1.1 -3.7	1.3 - 4
Monocytes	0.6 ± 0.1	0.3 – 0.8	0.5 ± 0.2	0.2 -1.1	0.2 -0.8
Neutrophils	3.8 ± 1.2	1.1 - 5.1	3.4 ± 1	1.6 – 5.6	2 – 7.5
Eosinophils	0.3 ± 0.2	0.03 - 0.65	0.3 ± 0.3	0.1 – 1.4	0.05 -0.5
Basophils	0.04 ± 0.03	0 – 0.11	0.03 ± 0.02	0 – 0.09	0 – 0.2

Results are expressed as unit x 10^3 / mm^3 , mean ± SD.

different, still could be considered to be at risk and should periodically undergo biological monitoring and appropriate care.

Conclusions

This study associates PM ($PM_{2.5}$ and PM_{10}), PAHs, and VOCs levels with oral mucosa genotoxicity. To this end, the frequency of nuclear aberrations in exfoliated oral mucosal cells from populations exposed to different levels of air quality was evaluated. It is noteworthy to mention that the population from the industrial area, exposed to a higher PM_{10} level ($p < 0.05$) and some VOCs like benzene, dichloroethane, and ethylbenzene showed no significant increases in MN percentage with respect to the urban population. This preliminary study, allowed us to recognize that individuals living in both areas could be considered as susceptible groups and should periodically undergo biological monitoring and appropriate care. Further studies with a larger sample of inhabitants should be done in order to confirm the data obtained herein.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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