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Genotoxic effects of daily personal exposure to particle mass and number concentrations on buccal cells

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GRAPHICAL ABSTRACT

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ABSTRACT

The aim of this study is to assess personal exposure to Particle Number Concentrations (PNC) in four size ranges between 0.3 and 10 µm, and particulate matter (PM_1; $PM_{2.5}$; PM_4; PM_{10}) in order to evaluate possible genotoxic effects through a comet assay in buccal cells. A convenience cohort of 30 individuals from a Brazilian medium-sized city was selected. These individuals aged between 20 and 61 and worked in typical job categories (i.e., administrative, commerce, education, general services and transport). They were recruited to perform personal exposure measurements during their typical daily routine activities, totaling 240 h of sampling. The 8-h average mass concentrations in air for volunteers ranged from 2.4 to 31.8 µg m^{-3} for PM_1, 4.2–45.1 µg m^{-3} for $PM_{2.5}$, 7.9–66.1 µg m^{-3} for PM_4 and from 23.1 to 131.7 µg m^{-3} for PM_{10}. The highest PNC variation was found for 0.3–0.5 range, between 14 and 181 particles cm^{-3}, 1 to 14 particles cm^{-3} for the 0.5–1.0 range, 0.2 to 2 particles cm^{-3} for the 1.0–2.5 range, and 0.06 to 0.7 particles cm^{-3} for the 2.5–10 range. Volunteers in the 'education' category experienced the lowest inhaled dose of $PM_{2.5,}$ as opposed to those involved in 'commercial' activities with the highest doses for PM_{10} (1.63 µg kg^{-1} h^{-1}) and $PM_{2.5}$ (0.61 µg kg^{-1} h^{-1}). The predominant cause for these high doses was associated with the proximity of the workplace to the street and vehicle traffic. The comet assay performed in buccal cells indicated that the volunteers in 'commerce' category experienced the highest damage to their DeoxyriboNucleic Acid (DNA) compared with the control category (i.e. 'education').

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These results indicate the variability in personal exposure of the volunteers in different groups, and the potential damage to DNA was much higher for those spending time in close proximity to the vehicle sources (e.g. commercial services) leading to exposure to a higher fraction of fine particles. This study builds understanding on the exposure of people in different job categories, and provide policy makers with useful information to tackle this neglected issue.

1. Introduction

Several studies carried out in the last decades have linked air pollution to human health damage, compromising the quality of life and the well-being of citizens (Pope, 2000; Brunekreef and Holgate, 2002; Chen et al., 2013). The airborne particulate matter (PM), which is one of the most harmful air pollutants, is associated with an increase in mortality rate, reduced life expectancy, increased hospital admissions and emergency room visits, asthma, chronic bronchitis, cancer, cardiovascular diseases, diabetes, among others (Brown et al., 1950; Dockery et al., 1992; Seaton et al., 1995; Coelho and Saldiva, 2011; Arbex et al., 2012; Pope et al., 2004; Li et al., 2008; Jung et al., 2012; Kim et al., 2015). The World Health Organization (WHO) claimed air pollution as carcinogenic in 2013 (Loomis et al., 2014). Therefore, it is important to evaluate personal exposure of urban dwellers with a high temporal resolution during their normal indoor and outdoor activities.

The PM chemical composition and size distribution vary in the atmosphere according to their emission sources, physical-chemical atmospheric conditions (Hildemann et al., 1991; Colbeck, 2014). When inhaled, they are selectively deposited throughout the respiratory tract according to their sizes. Therefore, the particle size is directly related to potential health damages, affecting the lungs and other organs (Reibman et al., 2003; WHO, 2006a,b; Ning et al., 2008).

Pollution monitors have evolved over the past decade and have become widely used to measure personal exposure, as well as being used in studies relating public health and air quality (Wallace et al., 1985; Ozkaynak et al., 1996; Williams et al., 2000; Gulliver and Briggs, 2004; Viet et al., 2013; Spinazzè et al., 2015). The use of these personal monitors, for example, provides the understanding of the real PM exposure levels in internal and external environments, as well as the assessment of the different degrees of exposure of the population. (Buonanno et al., 2014; Azarmi et al., 2014; Schiavon et al., 2015; Steinle et al., 2015; Hou et al., 2016). It is also essential to know the Particle Number Concentrations (PNC) since they vary substantially in urban environments (Kumar et al., 2011, 2014) and may even be more related to health hazards (Nel, 2005). In Brazil, there are only a handful of studies measuring particle concentration in the air using personal monitors (Göethel et al., 2014; Cozza et al., 2015). There is hardly any assessment of the exposure to PM concentrations and PNC of urban dwellers under real-world exposure scenarios that assess their potential damaging effects on human health.

In order to fill the existing research gap in the literature, the objective of this exploratory pilot study was to measure personal exposure to PM concentrations and PNC during typical workdays of healthy adult volunteers from a medium-sized Brazilian city. Furthermore, it also investigated the possible DeoxyriboNucleic Acid (DNA) damage associated with this air pollutant through the comet test in buccal cells and regression model.

The comet assay has been increasingly used as an indicator of genotoxicity for biomonitoring of occupational and environmental exposures (Albertini et al., 2000; Faust et al., 2004; Bates et al., 2005; Møller, 2006; Au, 2007; Carbajal-López et al., 2016). It is considered an

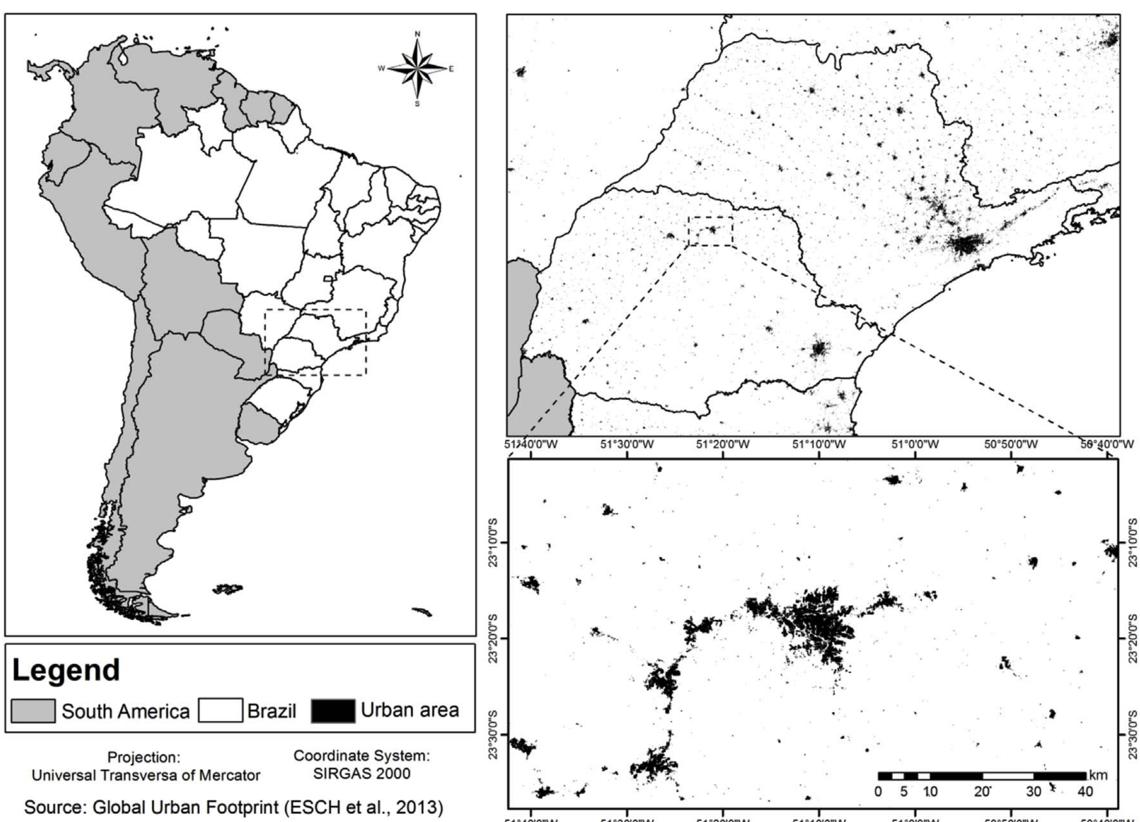

Fig. 1. Location of the study region, with emphasis on the distribution of the urban area.

inexpensive, simple, and sensitive technique for measuring primary DNA damage, and can provide information on risk assessment of environmental and occupational exposures (Sánchez-Alarcón et al., 2016).

The use of buccal cells is an indicator of genotoxic damages associated with exposure to pollutants. These damages can be explained by the ability of cells to metabolize genotoxic agents and to be one of the first physical barriers of the human respiratory tract (Sisenando et al., 2012). In addition, the mouth offers a good area of contact for particulate pollutants, and thereby the sampling of cells is simple and fast, and cause little discomfort to the volunteers.

It is important to emphasize that the focus was to understand the real-world exposure of urban dwellers during their work activities and movements in the urban area and their potential DNA damage. Moreover, it aimed at providing information to support the elaboration of public policies concerning the PNC and PM2.5, since they affect human health.

2. Methodology

2.1. Selection of volunteers

The study was conducted in the city of Londrina, in Brazil, which has approximately half a million inhabitants (IBGE, 2011). The geographical feature of Londrina is shown in Fig. 1. Cities such as Londrina (100,000 to 500,000 inhabitants) represented about 27% of the Brazilian cities for the year 2010. The city is growing fast in terms of its population and occupied area (Stamm et al., 2013) and, thus, there is also an increase in potential sources of pollutants such as vehicles and industries.

A convenient size sample of 30 volunteers from both genders agreed to participate in the study. They were from five occupational categories with 6 in each category: commerce, transport, administrative, education and general services, and were between 20 and 61. The category 'commerce' refers to shop keepers at commercial places. The 'transport' category refers to taxi drivers and bus drivers, while the 'administrative' category refers to office employees. Students, teachers and researchers are part of the education category whereas waiters, cleaners and bricklayers represent the 'general services' category. These occupation categories account for about 60% of the total active working class in this type of cities in Brazil.

Volunteers presented different exposure profiles due to their diverse daily routines. For example, they used different types of transport for commuting (bus, car, motorcycle or on foot). Their location and distances of the workplaces from their homes were different and the activities performed by them in indoor or outdoor environments were different. Moreover, they worked in different parts of the city including central and peripheral areas.

Participants were excluded if they met criteria for a history of smoking and alcohol consumption, or use of blood pressure medications, since these characteristics are considered immunocompromising, and the study should be performed on healthy volunteers. Additionally, a short questionnaire containing personal questions was filled by the volunteers. It contained information such as their name, sex, date of birth, age, home and business address, profession, means of transport and working hours. The study was approved by the Research Ethics Committee (protocol number 30166214.9.0000.5547).

2.2. Personal and environmental sampling

The sampling period was from August 25 to September 26, 2015, during workdays, with measurements performed for approximately 8 uninterrupted hours for each volunteer, starting from the moment they left their homes to go to work. The devices were placed in a small backpack, with the inlets externally attached to the strap near the volunteer's airways. They consisted of the following equipment: (i) GPS DG-100 Data Logger Global Sat, with 3-s temporal resolution; (ii) Particle Counter monitor (Met One Instruments, Model 804, Grants Pass, U.S.A) to measure the PNC for the selected size ranges of 0.3–0.5 ($PNC_{0.3\text{-}0.5}$), 0.5 to 1.0 ($PNC_{0.5\text{-}1.0}$), 1.0 to 2.5 ($PNC_{1.0\text{-}2.5}$) and 2.5–10 µm ($PNC_{2.5\text{-}10}$); and (iii) Mass Particle Monitor (Met One Instruments, Model Aerocet 831, Grants Pass, U.S.A.) in order to provide the PM concentrations in the diameter sizes of 1 µm (PM_1), 2.5 µm ($PM_{2.5}$), 4 µm (PM_4) and 10 µm (PM_{10}). The temporal resolution for both pieces of equipment was set for 1 min, with a flow operation of 2.83 L min^{-1}. The sampling took place over a typical working day for each volunteer, totaling 240 h, giving a sampling time of about 48 h for each category. Holidays and weekends were not considered.

In order to obtain a reference for the environmental concentrations of PM_{10} and $PM_{2.5}$ during the personal sampling, a Partisol 2000i-D (Thermo Scientific, USA) sampler with 47-mm quartz fiber filters QM-A and 2-µm pore size (Whatman, USA) was used. The sampler was installed at the Federal University of Technology – Paraná, on the Londrina University campus, and the particles were collected during 24 h at 15 L min^{-1} for a coarse fraction and 1.67 L min^{-1} for a fine fraction. The concentrations were calculated by weighing the filters before and after sampling. It is important to highlight that the region does not have air quality monitoring stations.

2.3. Particle inhalation dose

The average personal dose inhalation was calculated in the mass of particles per kg of weight body for an 8-h exposure time, and it was compared among the occupational categories. Equation (1) (U.S. EPA, 1997) was used for the calculation of the average hourly inhaled dose (μg kg^{-1} h^{-1}):

$$\text{Inhaled Dose} = \frac{(\text{Ca*IR*ED})}{(\text{BW*AT})}, \tag{1}$$

where: Ca is the PM_{10} or $PM_{2.5}$ average concentration in air in the sampling period (µg m^{-3}), IR is the inhalation rate (m^3 h^{-1}) of 0.96 m^3 h^{-1}, considering an average between sedentary and light activity (U.S. EPA, handbook 2011), ED is the exposure duration (8 h), BW is the volunteer's body weight (kg) and AT the average time that the measures were considered (8 h). Other pathways of intake were not evaluated.

2.4. Comet assay

The comet assay had the purpose of evaluating the potential genotoxic DNA damages caused by genotoxic agents. It is considered a suitable and fast test for DNA-damaging potential in biomonitoring studies (Collins et al., 2014). We collected two samples of buccal cells (before and after 8 h of exposure) by scraping the buccal mucosa using a sterile toothbrush for each volunteer. The alkaline comet assay was performed as described by Singh et al. (1988), with modifications suggested by Tice et al. (2000). The Damage Frequency (DF) and Damage Index (DI) were accounted in the cells in order to possibly associate the DNA damages with particle concentrations measured in all volunteers, and make a comparison among occupational categories. The DF indicates the occurrence of damage in the cells, whereas the DI indicates the intensity of damage. In other words, the DF is the number of cells with damage regardless of its degree of damage counted. As opposed to the DI, that is a measure of the damage intensity regardless of the frequency. It is important to measure both and compare the values among all categories for the reason that the differences in DF and DI can indirectly be related with size and chemical composition of particulate matter and their damaging effect of the DNA.

To analyze the differences between occupation categories in relation to potential DNA damage, Duez et al. (2003) proposed to perform the Kruskal-Wallis test on the DF and DI data. This test allows the comparison of individual groups of samples that have passed the comet test. Therefore, we performed the Kruskal-Wallis test to the DF and DI data to verify if the observed differences between the categories are statistically significant in relation to the control group (i.e. education). The significance was tested considering $p < 0.05$.

3. Results and discussion

3.1. Personal and environmental particle concentrations

During the sampling period, the average daily temperature was 23.2 °C, the average daily cumulative rainfall was 45.9 mm (precipitation occurred only in 3 days). The mean daily relative humidity was 68%. The wind speed did not exceed 1 m s^{-1} and the predominant direction in the 30 days of sampling was northwest. These observed meteorological characteristics are within the climatic normality for the period, according to the climatological analysis previously performed for the studied region.

Table 1 presents a statistic description of all data measured during the personal and environmental sampling periods. It is interesting to note that the mean and median values are different, indicating a large heterogeneity of data. The personal mass concentrations of PM_{10} and $PM_{2.5}$ reached elevated values when compared to those recommended by the WHO standard, which should be 50 and 25 μg m^{-3} for a period of 24-h environmental measures, respectively (WHO, 2006a,b). The personal exposure to PNC was on average higher in lower ranges, reaching more than 181 particles cm^{-3} for $PNC_{0.3\text{-}0.5}$. It is important to note that concentrations of PM_{10} and $PM_{2.5,}$ even below the WHO standards, can cause damage to human health (Harrison and Yin, 2000; Pelucchi et al., 2009; Pope and Dockery, 2006; Kim et al., 2015). For example, cardiopulmonary and lung cancer mortality has been observed at a 95% confidence in response to long-term exposure to concentrations lower than the WHO (2006a,b) standards. The average concentrations measured at a fixed place over the sampling period were 11.8 μg m^{-3} and 21.3 μg m^{-3} for $PM_{2.5}$ and PM_{10}, respectively, which are lower than those to which the volunteers were exposed, including indoor concentrations.

Fig. 2A and B presents the PNC during approximately 8 h of exposure for the volunteers with the highest and lowest concentrations observed among all volunteers, respectively. The PNC were higher in smaller sizes, even though the range sizes are not equally distributed. The concentrations in the 0.3–0.5 μm range size were much higher when compared to other size intervals. Some peaks were observed during the time the individuals were going to work and during work, which can be associated with times when the volunteer was closer to the source and thus, more exposed. The volunteer with the highest number concentration belongs to the 'general services' category, working as a bricklayer. A sharp peak around 10 h (local time) could be observed, especially for 2.5–10 μm size, indicating a high exposure to higher size particles in the activity carried out at this time. When the volunteer moved from home to the workplace, the PNC presented an oscillating behavior (Fig. 2A). When the individual was at work, smaller particles were present in a higher number, albeit stable, while the number concentration of larger particles fluctuated during the sampling period, which is consistent with his work activity. The volunteer who presented the lowest number concentrations (Fig. 2B) belonged to the 'education' category. The peak occurred at about 07:30 a.m., and it is associated with the moment that this volunteer entered a transport vehicle since a rapid displacement was recorded by the GPS. This observed peak suggests a great influence of vehicle emissions on the concentration of small particles. During the route taken by volunteers using a public bus, the largest PNC were recorded in smaller size ranges.

In order to better understand the behavior of these concentrations and the possible health hazards associated with them, it would be important to apply a questionnaire on the specific types of activities during the sampling period, analyzing if the volunteer remained longer in an open or in a closed environment, or if he was using a transport vehicle at that time.

Table 2 shows the average PNC and PM by studied categories. The 'commerce' category presented the highest average of PM and PNC for the smallest size ranges, which were of 180 particles cm^{-3} for $PNC_{0.3\text{-}0.5}$, and of 15 μg m^{-3} for PM_1. This is probably due to higher particle generation in places where the volunteers carried out activities for this occupation category, most of the time in the center of the city, where there is an intense flow of vehicles. Additionally, fine particles are transported and accumulated inside workplaces, and thus increasing the level of exposure (Wu et al., 2012; Miller et al., 2017). The 'transport' presented the largest mass concentrations in larger sizes, probably associated with the resuspension material from auto-related activity. However, in general, mass and number concentrations in the larger sizes did not present a significant difference among 'transport', 'administrative', and 'general services' categories. The lowest PNC and PM concentrations were presented by the 'education' category as expected. This was due to the fact that volunteers stayed most of the time inside University rooms, which are places with the lowest influence of industrial and vehicular sources.

The above results should be seen with caution since most of the PNC is observed in the ultrafine fraction (smaller than 0.1 μm), a range not sampled in this study. Therefore, the PNC is probably much higher than the values found here (181.4 particles cm^{-3} for $PNC_{0.3\text{-}0.5}$). However, this does not hinder the estimation of inhaled particle dose, which depends mainly on the mass attributable to particles in the size range above 0.1 μm.

The differences of PM and PNC among the occupation categories were examined to determine if these differences were statistically significant. The non-parametric Kruskal-Wallis test was applied for all particle sizes measured and the results, as well as the average concentrations for each category are shown in Fig. 3.

A significant difference was found for PM and PNC for all categories in relation to the control group 'education' (Fig. 3). The 'transport' category presented the greatest differences for PM and PNC. This was expected given that vehicular emissions in urban areas are responsible for much of the coarse concentrations (soil resuspension promoted by vehicles) and fine (mainly from heavy-duty vehicles) PM (Harrison et al., 1997; Martins et al., 2012; Kumar et al., 2016, 2017). Additionally, the influence of outdoor air in the indoor environment was observed for 'administrative' and 'commerce' categories, which were associated with the ingress of the external air, the proximity of the street with intense flow of vehicles and the inefficient removal inside offices or stores. These factors contributed to relatively high concentrations found in 'administrative' and 'commerce' categories. For the smallest particles, the 'commerce' category showed higher concentrations than those in 'transport. The largest differences were noted for concentrations in smaller particle size. In line with our results, Segalin

Table 1
Statistic description of data for variables measured in all volunteers and in a fixed place. PM in μg m^{-3} and PNC in particles cm^{-3}.

Variables	Minimum	Maximum	Median	Mean	Standard Deviation	C.V. (%)[a]
PM_1	2.4	31.8	11	12.9	7	54.3
$PM_{2.5}$	4.2	45.1	18.1	19.2	9.9	51.4
PM_4	7.9	66.1	25.5	29	14	48.4
PM_{10}	23.1	131.7	57.1	61.3	28.3	46.1
$PNC_{0.3\text{-}0.5}$	14	181.4	59.7	67.7	12.8	0.05
$PNC_{0.5\text{-}1.0}$	1.4	14.1	4.8	5.6	3.1	0.05
$PNC_{1.0\text{-}2.5}$	0.2	2.7	0.4	0.07	0.7	0.05
$PNC_{2.5\text{-}10}$	0.06	0.7	0.2	0.2	0.17	0.06
PM_{10} fixed	5.5	122.8	13.5	21.3	25.0	119.1
$PM_{2.5}$ fixed	1.0	94.2	8.4	11.8	17.2	145.9

[a] C.V. = coefficient of variation.

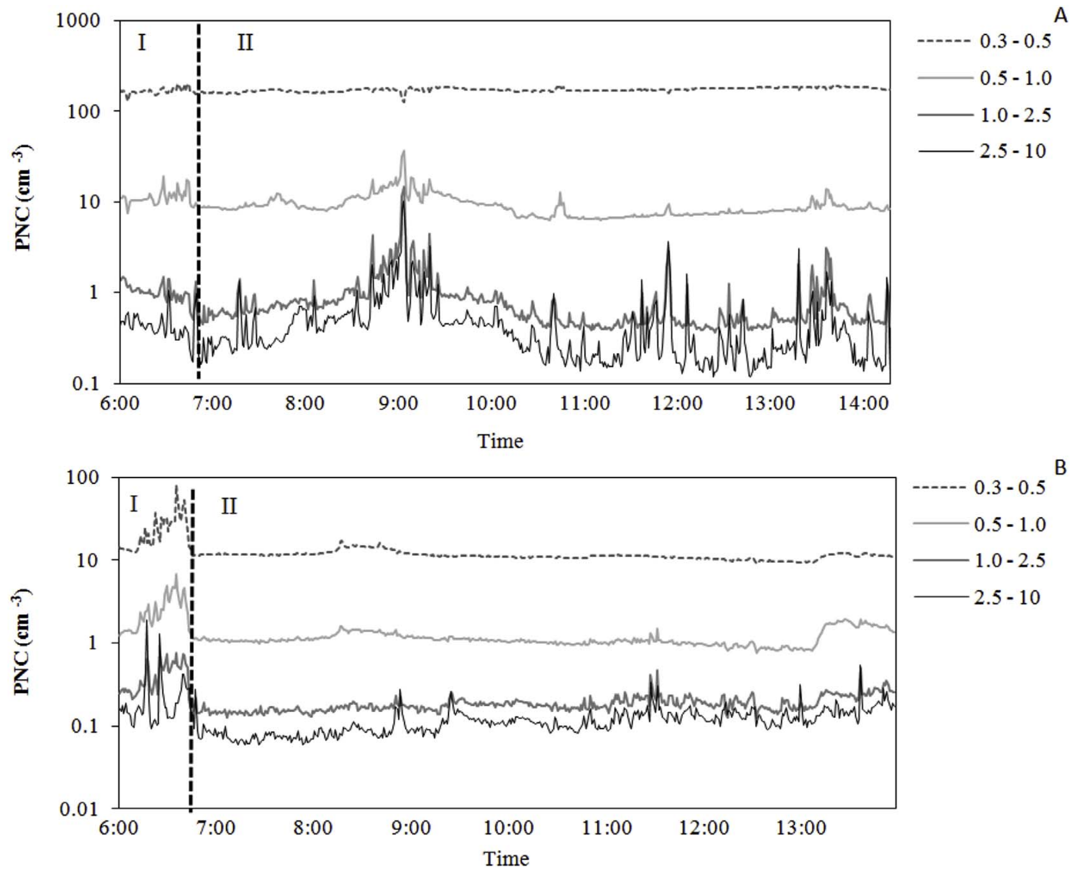

Fig. 2. PNC at four size ranges. (A) Volunteers with higher and (B) lower concentrations. I: going to work; II: at the workplace.

Table 2
Average PNC and PM by volunteers category in particles cm^{-3} and $\mu g\ m^{-3,}$ respectively.

Variables	Category				
	Administrative	Commerce	Education	General Services	Transport
PM_1	10.6 ± 2.1	15.5 ± 8.5	7.4 ± 3.3	14.3 ± 9.3	13.8 ± 3.9
$PM_{2.5}$	18.53 ± 6.1	20.9 ± 9.5	11.1 ± 4.1	21.5 ± 10.3	22.6 ± 13.1
PM_4	31.4 ± 13.0	28.5 ± 10.7	20.3 ± 8.71	31.7 ± 13.8	33.2 ± 19.6
PM_{10}	67.4 ± 27.7	50.9 ± 22.0	48.3 ± 14.8	57.8 ± 26.3	69.9 ± 36.6
$PNC_{0.3\text{-}0.5}$	59.6 ± 11.3	93.9 ± 55.4	39.9 ± 20.3	78.4 ± 46.1	77.1 ± 19.9
$PNC_{0.5\text{-}1.0}$	6.3 ± 1.7	7.4 ± 3.6	2.97 ± 1.2	6.3 ± 2.8	7.3 ± 4.4
$PNC_{1.0\text{-}2.5}$	1.7 ± 1.0	0.7 ± 0.3	0.3 ± 0.1	0.8 ± 0.5	1.3 ± 1.0
$PNC_{2.5\text{-}10}$	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.2

et al. (2017) also found higher concentrations of fine particles inside elderly residences located near areas with heavy traffic.

The concentrations presented large spatial variability during the volunteer's displacements. As an example, the route and the $PM_{2.5}$ concentrations from a volunteer in the 'commerce' category are presented in Fig. 4. This is a typical route used by this volunteer, but all the others in the work category present similar behaviors, staying approximately 8 h at the same place, near the streets. This representative volunteer from the 'commerce' category walked to work, in a region with an intense vehicle traffic. This route resulted in high $PM_{2.5}$ concentrations during displacements, with average and maximum concentrations of 34.6 $\mu g\ m^{-3}$ and 58.2 $\mu g\ m^{-3}$, respectively, during the sampling period. For the PM_{10} concentration, the average was of 50 $\mu g\ m^{-3}$ in the workplace. Along the route, the PM_{10} concentrations reached up to 150 $\mu g\ m^{-3}$.

Volunteers in the 'commerce' category are those who work in shopping stores, which are usually open and in close proximity to the streets. Hence, the emissions from vehicles directly influence these workplaces by the ingress of fine particles. Vehicles, mainly diesel motors, emit smaller particles (e.g., black carbon, metals, polycyclic aromatic hydrocarbons) in relatively large quantities than other urban sources. They also promote the re-suspension of particles from the ground, which are a mixture of fine and coarse particles. However, the coarse fraction deposits faster on to the road surface compared with fine fraction due to their larger settling velocity. In addition, the gases (SO_2, NOx, VOCs) emitted by urban sources (vehicles, bakeries, restaurants, etc) undergo complex physical-chemical processes such as nucleation (gas-particle process) that significantly contribute to the fraction of fine particles.

Table 3 shows a comparison between the measurements performed for different cities around the world with the average concentrations obtained in this study. Montagne et al. (2013) assessed fifteen volunteers from three European cities in order to compare their living quarters (traffic zone, urban and semi-urban area). It can be observed that the $PM_{2.5}$ concentrations are different among the cities. This can be justified due to the difference in the number of inhabitants, a different

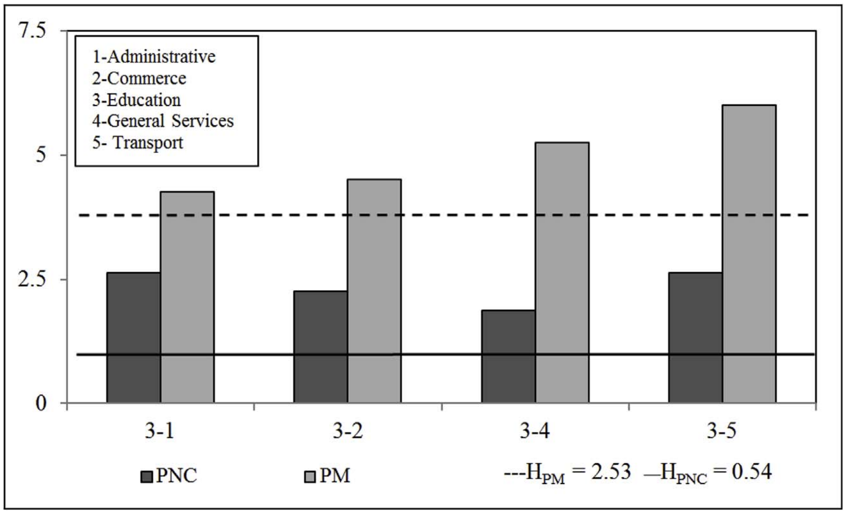

Fig. 3. Comparison of the average PM and PNC for all intervals and occupation categories in relation to control group 'education' category (3). Dashed lines indicate the critical difference threshold calculated in the Kruskal-Wallis test (p = .05). 3-1 means the comparison between 'education' and 'administrative' categories, 3-2 means the comparison between 'education' and 'commerce' categories, 3–4 means the comparison between 'education' and 'general services' categories and 3-2 means the comparison between 'education' and 'transport' categories.

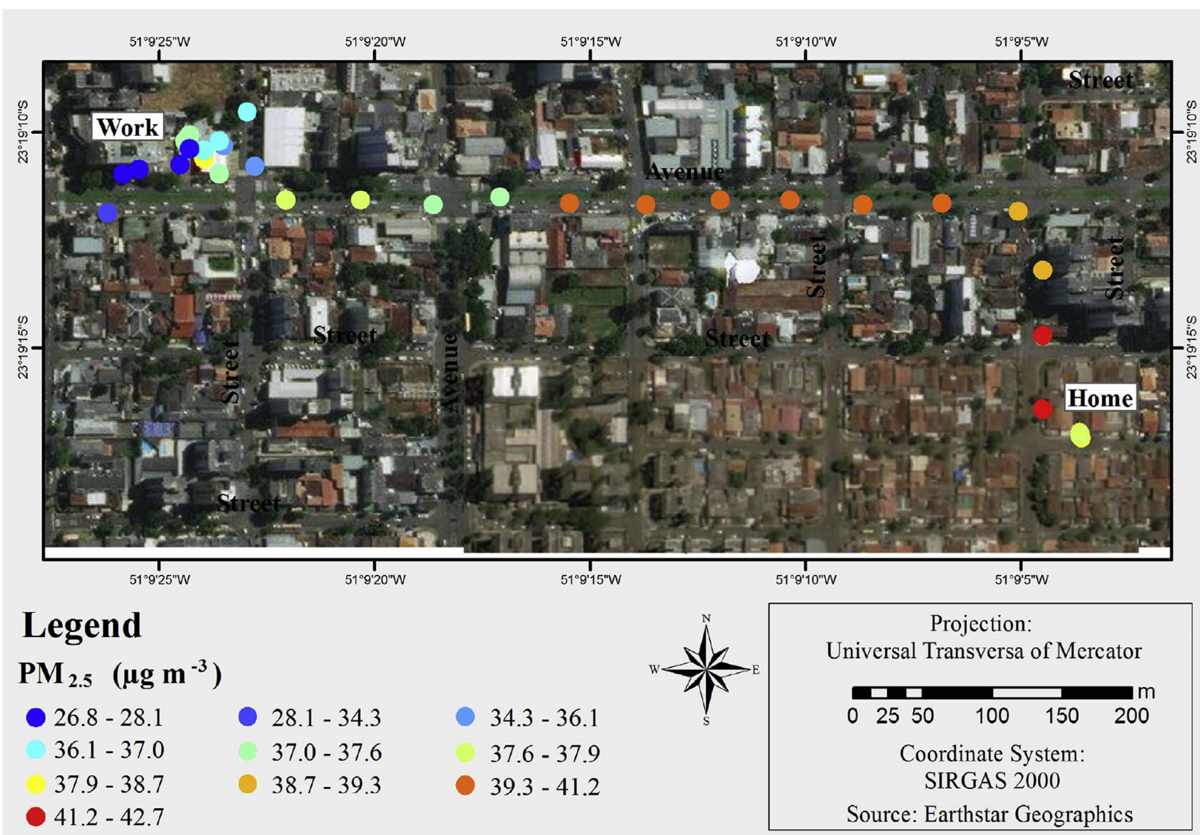

Fig. 4. Route traveled by a volunteer from the 'commerce' category.

Table 3
Comparison of $PM_{2.5}$ concentrations measured in different cities.

City	Period	Type	$PM_{2.5}$ (µg m^{-3})	Reference	Inhabitants
Bhubaneshwar, India	Dec. 2012–Feb. 2013	Stationary	60.7 ± 20	Panda et al. (2016)	837,737
Treviso, Italy	Dec. 2012–Feb. 2013	Stationary	44 ± 23	Squizzato et al. (2017)	884,353
Saint-Omer, France	Mar. 2011 – Ap.r 2011	Stationary	32.5 ± 17	Ledoux et al. (2017)	73,000
Edinburgh, United Kingdom	Sep. 1999–Sep. 2000	Stationary	7.1	Heal et al. (2005)	450,000
Ultrecht, Netherlands	Mar. 2010–Mar. 2011	Personal	11.2	Montagne et al. (2013)	335,089
Barcelona, Spain	Mar. 2010–Mar. 2011	Personal	21.7	Montagne et al. (2013)	1,619,000
Helsinski, Finland	Mar. 2010–Mar. 2011	Personal	6.4	Montagne et al. (2013)	629,512
Londrina, Brazil	Aug. 2015–Sep. 2015	Personal	19.2 ± 9.9	This work	553,393

configuration of the vehicle fleet, different public policies in relation to atmospheric PM emission sources, and differences in the type of equipment used in the studies. However, high $PM_{2.5}$ concentrations were registered in Londrina at specific times.

Studies performed by Panda et al. (2016), Squizzato et al. (2017) and Heal et al. (2005) show stationary source measurements of $PM_{2.5}$ in mid-size to large cities in Asia and Europe. In Bhubaneswar, the high concentrations were attributed to weather conditions in the sampling period (winter) and to the great local sources (biomass burning and industries). In Treviso, the authors identified six sources of particle emission: combustion, ammonium nitrate, ammonium sulfate, industrial, traffic non-exhaust and resuspended dust. Beyond the relation with combustion, in Edinburgh and France, the high concentrations found by the authors were associated to local meteorology and synoptic conditions that contributed to relatively high concentrations. Therefore, they can be considered as extreme events and do not represent adequately the average conditions. In Londrina, the average $PM_{2.5}$ concentration was of 19.2 $\mu g\, m^{-3}$, which is lower than those found in Asian cities, although higher than those found in European cities, even when compared with Barcelona, which is more populous than Londrina. However, these comparisons of $PM_{2.5}$ concentrations among cities should be analysed with caution, since the sample design in the works are different.

3.2. Inhaled dose

Fig. 5 shows a comparison of the average inhaled dose of particles among five categories of volunteers. The 'education' category presented the average lowest doses for $PM_{2.5}$ and PM_{10}, as opposed to the highest doses in terms of both the average and absolute values for the 'commerce' category. For the 30 volunteers, the average inhaled dose for PM_{10} was of approximately 0.77 $\mu g\, kg^{-1}\, h^{-1}$, while it was 0.24 $\mu g\, kg^{-1}\, h^{-1}$ for $PM_{2.5.}$ Zuurbier et al. (2010), for example, found higher dose values in their dose assessment study carried out in Arnhem, Netherlands. However, their measurements were performed exclusively in different transport modes, route and fuel, which, in general, are conditions with higher exposure in a short time. The inhaled dose of particles obtained in this study means an average inhaled dose considering the diversity of exposure conditions along voluntary workday, which are the real-world conditions people are usually exposed to in urban environments. Health effects associated with inhaled dose of particles may be higher when considering how variable the chemical composition can be and their toxicity, with metallic and organic compounds in their composition (Beal et al., 2017; Ham and Kleeman, 2011).

3.3. Comet assay

The groups with significant difference in the DNA damage (DF and DI), when compared to the control group, based on-parametric test results (H = 5.35 and 9.19), were 'commerce' and 'general services' as shown in Fig. 6. These results are steady, considering that these activities presented higher concentration of particles, mainly of fine particles for the 'commerce' group, which are known as the cause of more damage to human health. It is important to highlight that in the PM chemical composition, especially the fine of vehicular origin, there are chemical compounds such as polycyclic aromatic hydrocarbons, toxic metals, black carbon, which are known to be toxic and/or genotoxic to humans.

The 'transport' category, differently from what was expected in the beginning of this work, did not present significant differences in relation to the control group. In this group, three volunteers worked as taxi drivers and drove around the city with windows up and air conditioning on. Probably for this reason, the DNA damage, as well the doses found for this occupation were lower when compared to 'commerce' category, which presented the highest doses and DNA damage in relation to control.

3.4. DNA damage versus particles concentrations

Regression analysis was performed to analyze the possible DNA damage associations with particle concentrations (Wiencke et al., 1999). A binomial negative model presented the best fit with lower values of Akaike criterion (Bozdogan, 1987; Posada and Buckley, 2004). The DF and DI to the moment before and after the sampling are statistically not similar, based on answers from the Shapiro-Wilk and Mann-Whitney tests. The DF and DI accounted for a moment before we assumed are from long-term exposure and other agents. Therefore, the regression analysis was performed with DF the moment after exposure was measured and with the variations (DF after minus DF before). This was chosen as a response, since it relates more directly the occurrence of DNA damage to the measured pollutants.

The independent numerical variables, which are: $PM_{1.0}$, $PM_{2.5}$, PM_{10}, $PNC_{0.3\text{-}0.5}$, $PNC_{0.5\text{-}1.0}$, $PNC_{1.0\text{-}2.5}$ and $PNC_{2.5\text{-}10}$, and the inhaled doses of PM_{10}, were tested in a univariate model. The Relative Risk (RR) and their Confidence Intervals (CI) can be observed in Table 4. The variable Occupation Category was taken from the multivariable model, even showing association with the response because its RR was outside the CI, showing great data heterogeneity.

The results indicated associations with the smallest particle diameters for PNC and PM concentrations (PM_1, $PM_{2.5}$ and $PNC_{0.3\text{-}0.5}$), as

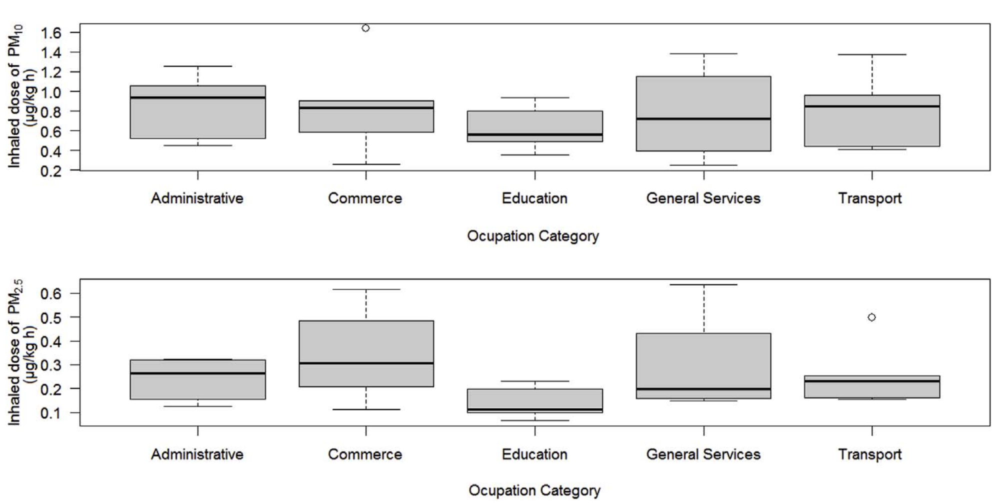

Fig. 5. Boxplot chart presenting the calculated average doses inhaled distributed according to the occupation categories. The line in bold represents the median, the two horizontal lines are the first and third quartiles, the bars in the top and below represent the maximum and minimum values, respectively, and the points are the outliers.

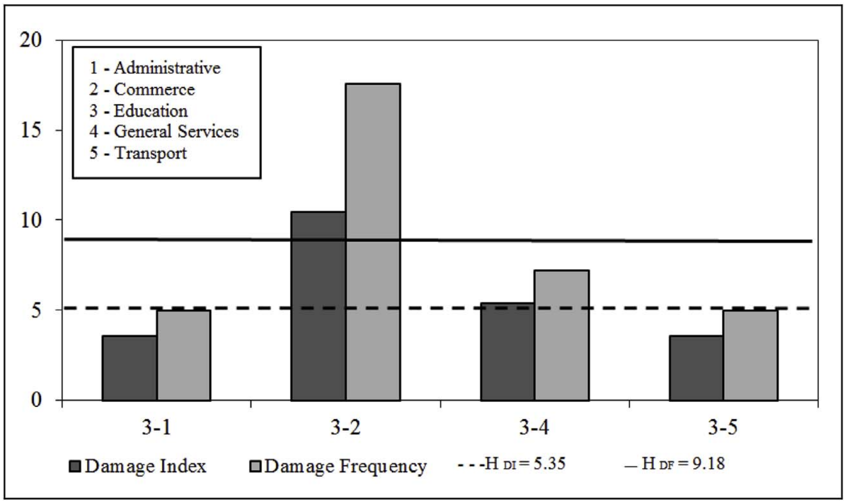

Fig. 6. Comparison of the Damage Frequency and Damage Index in buccal cells after exposition for occupation categories. Dashed lines indicate the critical difference threshold calculated in the Kruskal-Wallis test (p = .05). The 3-1 means associations between 'education' with 'administrative' category, 3-2 means associations between 'education' with 'commerce', 3–4 means associations between 'education' with 'general services' and 3-2 means associations between 'education' with 'transport'.

Table 4
Relative Risk (RR) calculated for the independent variables associated to the response variables Damage Frequency (DF) and ΔDamage Frequency (ΔDF) in the univariate and multivariate models with p = .05.

	DF			ΔDF		
Univariable	Variable	RR	CI*	Variable	RR	CI
	PM1	1.19	(-0.93 - 1.29)	PM1	1.02	(-12.85 - 12.84)
	PM2.5	1.12	(-0.92 - 1.15)			
	PNC0.3–0.5	1.00	(-1.63 - 1.63)			
Multivariable	PNC1.0–2.5	1.26	(-6.48 - 6.94)			

CI = Confidence Interval, coefficients Δ in the confidence intervals calculation for the numerical variables: 5 particles cm^{-3} to $PNC_{0.3\text{-}0.5}$ and $PNC_{1.0\text{-}2.5}$; 10 $\mu g\,m^{-3}$ to $PM_{2.5}$ and; 7 $\mu g\,m^{-3}$ to $PM_{1.0}$ concentrations.

expected, and agreeing that smaller particles may have more health damage. The all day long exposure to air pollution increases DF and DI index after 8 h of exposure. These results are consistent with the all day long increase of $PM_{2.5}$ concentrations and explain its association with occupational category 'commerce', which present the highest DNA damage and doses.

4. Conclusion and final considerations

The results of this study show that it is feasible to carry out a personal sampling of air pollutants under different exposure conditions, although human and financial resources are important limiting factors.

The highest concentrations in number occurred while the volunteers were moving through the streets, which was related to vehicle emissions. In average, the category with the highest inhaled dose of PM_{10} and $PM_{2.5}$, and the DNA damage was 'commerce', an activity that presented the greatest exposure to higher concentrations of fine particles, which can be explained by the proximity of shops to streets and vehicular emissions and the open design of the buildings. Additionally, the comet assay indicated that smaller particles may cause more DNA damage.

Finally, this study builds understanding on people's exposure, in different job categories, providing useful information for decision makers, aiming at tackling people's exposure to PNC and $PM_{2.5}$ and its damage to human health. Besides, it will assist to attract the attention of the governmental authorities to this neglected issue.

Further studies are recommended using a larger group with more homogeneous characteristics (weight, gender, age) in order to represent workers in typical Brazilian cities. Besides, the PM chemical composition needs to be investigated in the future.

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