Characterization of the particulate matter and relationship between buccal micronucleus and urinary 1-hydroxypyrene levels among cashew nut roasting workers*

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A B S T R A C T

The present study is the first assessment of occupational risk associated with artisanal cashew nut roasting using exposure and effect biomarkers, as well as a characterization and dispersion analysis of the released particulate matter (PM). A real-time particle monitor was used to quantify PM_{1.0}, PM_{2.5} and PM_{10}. Furthermore, the PM was sampled using a Handi-vol sampler, and the physicochemical characteristics were determined by SEM-EDS analysis. Trajectories, dispersion and deposition of the emitted material were calculated using the NOAA-HYSPLIT model. Urinary 1-hydroxypyrene (1-OHP) levels were analyzed by HPLC. DNA damage, chromosomal instability and cell death were measured by a buccal micronucleus cytome assay (BMCyt). The PM concentrations for all measurements in the exposed area were higher than in the non-exposed area. SEM-EDS analyses exhibited a wide variety of particles, and K, Cl, S and Ca biomass burning tracers were the major inorganic compounds. In addition, atmospheric modeling analysis suggested that these particles can reach regions farther away than 40 kilometers. Occupational polycyclic aromatic hydrocarbon exposure was confirmed by increases in 1-OHP levels in cashew nut workers. Frequencies of BMCyt biomarkers of genotoxicity (micronuclei and nuclear bud) and cytotoxicity (pyknosis, karyolysis, karyorrhexis and condensed chromatin) were higher in the exposed group compared with the controls. The influence of factors, such as age, on the micronuclei frequencies was demonstrated, and a correlation between 1-OHP and micronuclei was observed. To the best of our knowledge, no other study has demonstrated a correlation between these types of biomarkers. The use of exposure (1-OHP) and effect (BMCyt) biomarkers were therefore efficient in assessing the occupational risk associated with artisanal cashew nut roasting, and the high rates of PM_{2.5} are considered to be a potential contributor to this effect.

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1. Introduction

Artisanal cashew nut roasting is an important economic and social activity throughout the world. However, the largest producers of cashew nuts are in developing tropical countries that do not offer basic conditions for the sustainable development of this activity. According to the Food and Agriculture Organization of the...
United Nations, approximately 31 countries worldwide produced 4.15 million metric tons of cashew nuts in 2012. The ten major cashew nut producing countries are Vietnam, Nigeria, India, Ivory Coast, Benin, Philippines, Guinea-Bissau, Tanzania, Indonesia and Brazil (FAOSTAT, 2012).

One of the main problems in artisanal cashew nut manufacturing is the conditions under which roasting takes place to obtain the kernel. The nuts are oven-roasted, where the shell waste itself acts as the fuel (De Oliveira Galvão et al., 2014). The released cashew nut shell liquid (CNSL) contains highly flammable caustic phenolic oil (Agila and Barringer, 2011; Chandrasekara and Shahidi, 2011; Liatrotakoon et al., 2016). Recently, the shell waste from cashew nut processing has been proposed as an alternative larvicide against the dengue vector Aedes aegypti (Torres et al., 2015). The air pollutants generated during the roasting process are inhaled for periods that may exceed 10 h per day. In Brazil, this generally occurs between 2:00 a.m. and 12:00 p.m., and it releases several air pollutants, especially particulate matter (PM) (De Oliveira Galvão et al., 2014).

We have previously reported that airborne particles from cashew nut roasting generated genotoxic effects in a plant model (Tradescantia Pallida), probably due to the high presence of mutagenic and carcinogenic [benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno (1,2,3-c,d)pyrene and dibenz(a,h)anthracene] polycyclic aromatic hydrocarbons (PAH) adsorbed on the PM surface (De Oliveira Galvão et al., 2015). Similar results were obtained by De Oliveira Alves et al. (2014) for biomass burning derived from the Amazon forest.

Occupational exposure to PM can exceed ambient levels. Due to increased interest in the health effects of PM, a variety of particle sampling methods have been developed such as the gravimetric sampling method, inertial mass measurement and optical methods (Moosmiller et al., 2001; Kim et al., 2004).

A direct-reading instrument, such as the DustTrakTM Aerosol Monitor, were well-correlated and highly predictive of gravimetric PM2.5 concentrations when sampling welding fumes and residual oil fly ash (Kim et al., 2004); biomass fuel smoke in domestic work (Kurmi et al., 2008); winter wood- and coal-smoke air pollution (Kingham et al., 2006); indoor university air (Yanosky et al., 2002) and indoor/outdoor areas in community residential areas (Ramachandran et al., 2000). The techniques used by real-time aerosol monitors provide information on the temporal pattern of the exposures, continuously collecting measurements over a short period of exposure time. One of its major advantages is ease of use. No additional analyses are required to obtain the PM concentrations, and the measurements can be recorded in an internal database. However, aerosol measurements with different optical properties tend to be overestimated (Ramachandran et al., 2000; Yanosky et al., 2002; Kim et al., 2004; Kingham et al., 2006).

Information on accurately measured airborne pollutants and understanding the interactions of these pollutants with climatological factors are fundamental when accounting for dynamic atmospheric studies. It has been recognized that air pollution is not only a local but also a regional problem. Some pollutants can be transported regionally (Wang et al., 2010; Rolph et al., 2014; Vellingiri et al., 2015), and even inter-continentally, depending on weather, orographic conditions and emission sources. Therefore, an important tool is the use of air dispersion models to simulate the trajectories, deposition and transport of air pollutants. Some of the applications of these models include forecasts of wildfire smoke, volcanic ash, windblown dust and atmospheric dispersion products for nuclear accidents and various chemicals (Rolph et al., 2014).

High exposures to PM in occupational settings are associated with significant increases in cancer risk to humans. The binding of PAH metabolites to DNA and the consequent effects is considered the main mechanism of PAH-induced carcinogenesis (Jarvis et al., 2014; Zhang et al., 2016). Pyrene is metabolized by CYP1A1 to 1-hydroxypyrene (1-OHP), which is excreted in the urine as the corresponding glucuronide. The 1-OHP quantification in urine gives a more accurate assessment of the total PAH exposure from all exposure routes than the PAH levels in air, including oral and dermal absorptions (Jongeneelen, 2001; Boström et al., 2002).

Several studies have demonstrated that urinary 1-OHP levels are an appropriate exposure biomarker for PAH in different environmental and occupational settings (Hansen et al., 2008; Miao et al., 2015; Yuan et al., 2015) and revealed that it is well-correlated with biomarkers of genotoxic effects in blood cells (Leng et al., 2004; Duan et al., 2009; Jasso-Pineda et al., 2015).

Buccal micronucleus cytome assay (BM cyt) have been extensively applied in studies of occupational populations exposed to genotoxic compounds. This technique has been widely used as buccal cells can be collected in a minimally invasive procedure (Holland et al., 2008; Thomas et al., 2009). In 2011, an inter-laboratory group compiled and analyzed BM cyt biomarkers from 5424 subjects using data from 30 laboratories worldwide to reduce protocol variability, assess the role of confounders and estimate a range of reference values for spontaneous buccal cell micronuclei (MN) was published (Bonassi et al., 2011).

BM cyt is used to measure biomarkers of chromosomal breakage and/or loss (micronuclei), indicative of gene amplification (nuclear bud), cytokinesis-failure or arrest (binucleated cells), proliferative potential (basal cells) and various forms of cell death (condensed chromatin, pyknotic, karyolytic and karyorrhectic cells). MN frequency has been associated with increased risk of cancer, neuro-degenerative diseases and accelerated aging (Bonassi et al., 2011; Bolognesi et al., 2013).

Due to this exposure, the aims of the current study were: i) to perform a characterization, quantification, trajectory, dispersion and deposition modeling of the emitted PM, and; ii) to assess the occupational risk associated with artisanal cashew nut roasting by measuring the surrogate of PAH exposure biomarker (urinary 1-OHP levels) and the biomarker of cytogenetic effect (BM cyt) in exposed and non-exposed groups.

2. Materials and methods

2.1. Particulate matter monitoring

The airborne PM concentration of interest involved four fractions: total suspended particles (TSP); thoracic or course (PM10), with aerodynamic diameter < 10 µm; respirable or fine (PM2.5), <2.5 µm; and (PM10), <1.0 µm. A TSI DustTrak™ II Aerosol Monitor (Model 8530, TSI, Inc.), with a laser diode photometer using 90° light scattering, equipped with 1 µm, 2.5 µm and 10 µm cut-off inlet was used to measure real-time PM emissions. This direct-reading instrument displays its measurement as mass density (i.e., units of mg/m3) with a resolution of ±0.1% reading of 0.001 mg/m3, recording the concentration at a flow-rate of 1.71 L/min at 10 s intervals in its internal data logger (TSI Inc, 2010). The monitoring campaign was conducted during February, March and May of 2015 in the Amarelão community (a region of intense cashew nut roasting ~ 05°30’51’’S, 35°54’17.13’’O) and Bioscience Center of the Federal University of Rio Grande do Norte – UFRN/CB (without the influence of the cashew nut processing ~ 05°50’28.8’’S, 35°12’07.4’’W). The exposed area was not directly affected by vehicular emissions or industrial pollutants. The characteristics and geographical information of the Amarelão community were described by De Oliveira Galvão et al. (2014). The equipment was positioned at the two test sites at a mean height of 1.60 m that approximately corresponded to the
height at which the workers breathe. The data were analyzed using TrakProTM v4.6.10 software.

2.2. TSP sampling

TSP were sampled according to an adaptation of the Brazilian technical standards for PM collection (ABNT-NBR 9547, 1997). Samples were only collected from the exposed area during February, March and May of 2015 using a Handi-vol sampler (Energética, Brazil) operated at a flow rate of 230 L/min, with a quartz fiber filter (EQTZ diameter of 110 mm, Energética, Brazil). The equipment was positioned at the same position of the direct-reading sampler (DustTrak™ II). The PAH analysis of these filters was previously reported by De Oliveira Galvão et al. (2015).

2.3. Physical and chemical characterization of TSP

The particles were characterized in terms of mass concentration, morphology, particle size distribution (PSD), and elemental composition by using gravimetric analysis and scanning electron microscopy (SEM) coupled with energy-dispersive x-ray spectrometry (EDS). The filters were analyzed using a Hitachi TM-3000 SEM fitted with SwiftFED3000 EDS. The technique allows for analyzing the microstructure, as well as the chemical composition of individual aerosol particles. Each filter was aligned to the center and a high contrast was used to distinguish them. The PSD analyses were carried out according to Huertas et al. (2012) and were manually determined by measuring the diameter of randomly selected particles in micrographs at various magnifications. Five points on the six filter sample were randomly selected and micrographs were acquired for each point at 1000×, 2000× and 3000×. EDS analysis of the filters was only performed qualitatively to observe if the particles were from cashew nut roasting, as the elemental composition was defined in previous studies with PM2.5 (De Oliveira Galvão et al., 2014). The results were affected by the presence of the quartz fiber filter (SiO2). When particles contain Si or O2, this technique cannot be used to determine the quantitative composition of the particles (Huertas et al., 2012). In this sense, the identification of these elements was not considered in this study.

2.4. The modeling system for trajectories, dispersion and deposition of particulate matter

A HYSPLIT-4.0 model (Hybrid Single-Particle Lagrangian Integrated Trajectory - version 4.0) was used for analysis of the trajectories, height, deposition area and dispersion of PM2.5 measured in the Amarelao community, developed by National Oceanic and Atmospheric Administration (NOAA), available at https://ready.arl.noaa.gov/index.php and described in detail by Draxler and Hess (1997) and Draxler et al. (2014).

The grid concentration was defined by the latitude-longitude intersection and we used the meteorological reanalysis data of National Center for Environmental Prediction/National Center for Atmospheric Research (NCEP/NCAR). This reanalysis data has a horizontal resolution of 2.5° of longitude-latitude and a vertical resolution of 17 levels, ranging from 10 hPa to 1000 hPa. To assess the HYSPLIT simulations with reanalysis data, climatological normal winds 10 m from the surface were used for the study region. The normals used in this work are historical averages from January 1, 1961 to December 31, 1990, corresponding to 394 weather surface stations from Brazilian National Institute of Meteorology (INMET), distributed throughout Brazil (Fig. S1). Air mass transport, deposition and dispersion were simulated with HYSPLIT during days with available measurements using the TSI DustTrak™ instrument in the artisanal cashew nut area.

Sampling of PM2.5 took place between 09:08 h – 10:08 h on February 27 (UTC-03:00); on March 2 from 10:29 h - 11:29 h (UTC-03:00); and on March 4 from 07:58 h - 08:58 h (UTC-03:00), of the year 2015. The simulations shown in this article are of those from February 27, since this day is representative of all the others. The simulations related to the other days are in the supplementary materials (Figs. S2 and S3).

2.5. Study population

This study was approved by the Brazilian National Committee on Research Ethics – Comissão Nacional de Ética em Pesquisa – CONEP (CAAE number 38708214.8.0000.5537) and written informed consent was obtained from each individual before the research began. A total number of 193 individuals aged 18~77 were randomly selected for this study. Exclusion criterion were being an active smoker and for women according the menstrual cycle status. No samples from women were collected during the menstrual period. They were divided into two groups: the exposed group was composed of 77 workers directly exposed to the artisanal cashew nut roasting pollutants. All of the exposed group samples were collected from the Amarelao community (exposed area). The non-exposed control group consisted of 116 individuals from UFRN/CB (control area) with no known occupational exposure to genotoxic agents, including air pollution from cashew nut roasting, coal, radiation, pesticides or dyes. Of this total, 40 subjects of the exposed group and 48 of the control group simultaneously provided urine samples and exfoliated buccal mucosa cells for correlation analysis of these biomarkers.

To characterize the study participants, they were asked to provide information from a Portuguese adapted questionnaire from the International Commission for Protection against Environmental Mutagens and Carcinogens (Carrano and Natarajan, 1988). The exposed and control groups were characterized by gender, age, alcohol consumption, family history of cancer, abortion and congenital anomalies.

2.6. 1-Hydroxypyrene (1-OHP) quantification

Urine samples were collected from each subject in a 50 mL urine collection tube. After sampling, the urine specimens were stored at −20 °C and light protected until further analysis. The analysis of 1-OHP in urine was an adaptation of the method previously described by Jongeneelen (2001). First, 2.5 mL of each urine sample were diluted with 5 mL of 0.1 M acetate buffer (pH 5.0) and treated for enzymatic hydrolysis using 4 µL of β-glucuronidase/arylsulfatase (Merck Millipore®). Then, the mixture was incubated at 37 °C for 16 h under continuous agitation. Urinary 1-OHP hydrolysate was extracted and purified by a solid phase extraction (SPE) procedure, in which 500 mg C18 cartridges (Bond Elut®, Agilent, CA, USA) were equilibrated with high purity methanol (2 mL) and ultra-pure water (2 mL); the retained solutes were eluted using 2 mL of methanol and then evaporated under a gentle nitrogen stream. The residue was re-suspended with 2 mL of methanol under sonication for 4 min to complete dissolution. After centrifugation (1400 rpm for 5 min), the clear extract was transferred to the HPLC auto-sampler vial. Urinary 1-OHP was determined by a high-performance liquid chromatography system (Agilent® 1260 infinity, Santa Clara, CA, USA) equipped with a fluorescence detector (PerkinElmer series 200, Shelton, CT, USA) using a reverse-phase C18 column (150 × 4.6 mm, 5 µm, Zorbax Eclipse XDB-C18, Agilent). The excitation and emission wavelengths were 232 nm and 396 nm, respectively. A 20-µL sample was injected into the column and eluted at a flow of 1.25 mL/min with a 70% methanol mobile phase.

The samples were processed in duplicate for each individual and
a 5 μg/L spiked urine sample was analyzed as a quality control in each run. Under our conditions, the method detection limit was 0.05 μg/L. The urinary 1-OHP levels were adjusted for urine creatinine levels measured by the Jaffe reaction (Tauskky, 1954). According to the World Health Organization’s (WHO) recommendations for valid urine specimens in occupational biomonitoring, samples with urinary creatinine levels below 30 mg/dL or above 300 mg/dL were excluded from data analysis (WHO, 1996). The urinary 1-OHP concentrations were expressed in μmol/mol creatinine.

2.7. Buccal micronucleus cytome assay (BMCyt assay)

The BMCyt was performed according to Thomas et al. (2009) with some modifications as described below. Initially, the subjects were asked to rinse their mouths with water before sampling and the exfoliated buccal mucosa cells were removed from each volunteer by gently rubbing in rotated action against the inside of the cheeks (right and left side) with a cytobrush. The collected samples were transported to the laboratory and kept in an upright position under refrigeration at a temperature of 4°C for 1 day. The cells were centrifuged three times at 1500 rpm for 10 min at room temperature and the buccal buffer was replaced for each centrifugation cycle. This procedure helped to inactivate endogenous DNAseases present in the oral cavity and to remove bacteria and cell debris that could disturb assessment (Thomas et al., 2009). After the last centrifugation, the pellet was fixed (ethanol: glacial acetic acid, 3:1 v/v) for 10 min. The suspended cells were dropped onto the labeled microscope slides, and allowed to air-dry. Fixed slides were treated for 1 min each in 50% (v/v) and 20% (v/v) ethanol and washed using deionized water for 2 min before staining. After drying, the cells were hydrolyzed in HCl (5 M) for 30 min. Staining was performed using the Schiff’s reagent for 60 min in the dark at room temperature. Afterwards, the microscope slides were immersed in 0.2% (wt/vol) Light Green for 20–30 s and rinsed well in deionized water.

BMCyt biomarkers of DNA damage (total number of micronuclei and nuclear buds), cytokinetic defects (binucleated cells), proliferative potential (basal cells) and cell death (pyknosis, karyorrhexis, and nuclear buds), cytokinetic defects (binucleated cells), proliferative potential (basal cells) and cell death (pyknosis, karyorrhexis, and nuclear buds) were classificed according to Bolognesi et al. (2013). The count was conducted under an optical microscope (Olympus CX31RBSFA) at 1,000× magnification. Two thousand cells for micronuclei and nuclear bud and one thousand cells for the other biomarkers were counted for each individual and the results are presented as the number of BMCyt biomarkers per thousand cells (‰).

2.8. Statistical analysis

Analysis of categorical variables (i.e., gender, alcohol consumption, family history of cancer, abortion, congenital anomalies and age) among the study population was performed using chi-square and Mann-Whitney tests. Kruskall-Wallis and Mann-Whitney tests were performed on PM monitoring data, 1-OHP quantification and the BMCyt biomarkers. Furthermore, the correlations among BMCyt endpoints, MN versus 1-OHP levels and roasting working time were evaluated by linear regression analysis followed by Spearman’s correlation analysis. Significant differences were considered significant at p < 0.05.

3. Results

3.1. Real-time particulate matter monitoring

Measurements of PM1.0, PM2.5, PM10 and TSP obtained for control and exposed sites using the real time monitoring are shown in Table 1. All measurements of PM were found to have higher concentrations for the Amarelão community compared to the control area (p < 0.0001).

3.2. Analysis of the TSP accumulated in the filters

Gravimetric analysis revealed an average concentration of 467 ± 206 μg/m3, and the mean for the particle size distribution was 7.3 ± 4.8 μm (range 1.09–28.9; Fig. 1A). Cumulative percentage analysis of the particle size distribution showed that 80% of the TSP presented a diameter less than 10 μm (Fig. 1B). Fig. 2 shows a series of photomicrographs with a wide variety of irregular particles in the sample. Spherical particles named tar balls (Fig. 2B); minerals, such as the quartz (Fig. 2D); and smooth-surfaced and cubic forms (Fig. 2E) were identified. In some cases, the particles were present as aggregates (Fig. 2C). EDX analysis indicated that the main chemical elements present in the particles were potassium, chlorine, sulfur, calcium and iron, corroborating our previous data on elemental composition in PM2.5 (De Oliveira Galvão et al., 2014). PAH analysis of these filters was previously reported by De Oliveira Galvão et al. (2015), with benzo[a]pyrene, benzo[k]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]pyrene and dibenz[a,h]anthracene being the most abundant PAHs.

3.3. Dispersion and deposition of the emitted particulate matter

It can be observed in Fig. 5 that the wind conditions in the State area were mainly driven by the annual cycle of the position and

| Table 1 |
| Measurements of particulate matter (PM) obtained using real time monitoring (DustTrak Aerosol Monitor) for the control (UFRN/CB) and exposed (Amarelão Community) sites. |

<table>
<thead>
<tr>
<th>Site</th>
<th>Particle</th>
<th>n</th>
<th>Minimum (μg/m³)</th>
<th>Maximum (μg/m³)</th>
<th>Median (μg/m³)</th>
<th>Mean (μg/m³)</th>
<th>S.D. (μg/m³)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFRN/CB</td>
<td>PM1.0</td>
<td>120</td>
<td>11</td>
<td>14</td>
<td>11</td>
<td>11</td>
<td>0.67</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PM2.5</td>
<td>240</td>
<td>16</td>
<td>20</td>
<td>18</td>
<td>18</td>
<td>0.77</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PM10</td>
<td>240</td>
<td>19</td>
<td>29</td>
<td>22</td>
<td>21</td>
<td>1.36</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>TSP*</td>
<td>120</td>
<td>22</td>
<td>41</td>
<td>25</td>
<td>25</td>
<td>2.48</td>
<td>–</td>
</tr>
<tr>
<td>Cashew nut roasting</td>
<td>PM1.0</td>
<td>1080</td>
<td>3</td>
<td>41,200</td>
<td>55</td>
<td>486</td>
<td>2678.2</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>PM2.5</td>
<td>1080</td>
<td>10</td>
<td>65,900</td>
<td>108</td>
<td>698</td>
<td>3734.0</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>PM10</td>
<td>1065</td>
<td>8</td>
<td>56,300</td>
<td>57</td>
<td>523</td>
<td>3433.8</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>TSP*</td>
<td>1080</td>
<td>7</td>
<td>44,800</td>
<td>87</td>
<td>483</td>
<td>2006.0</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

PM1.0 particles with aerodynamic diameter ≤ 1 μm.
PM2.5 particles with aerodynamic diameter ≤ 2.5 μm.
PM10 particles with aerodynamic diameter ≤ 10 μm.
TSP - Total Suspended Particulate.
UFRN/CB - Bioscience center of the Federal University of Rio Grande do Norte.
n - Number of measurements.
a - Significant difference compared to the UFRN/CB (Mann-Whitney test).
intensity of the Intertropical Convergence Zone (ITCZ) and the strong sea breeze. By analyzing climatic normals for the region, it is noted that there was not a significant variation of the wind throughout the year.

The winds were prevailing from the southeast and the direction varied a little between northwest and west, leading to an almost constant transport of the released PM$_{2.5}$ from the Amarello community in the same direction. Fig. S1C presents a simulation with wind reanalysis data from NCEP/NCAR for February 27, 2015. This complied with Fig. 3, which showed the PM$_{2.5}$ trajectory and height.

Fig. 3A shows that most of the particles remained below a height of 500 m after 2 h of simulation and at a distance of 10 km from the emission source. Fig. 3B shows the trajectory of the particles after 4 h of simulation, where most of them have already reached a height between 500 m and 750 m and are only 30 km away from the source. After 6 h (Fig. 3C), a greater quantity of particles reached a height between 750 m and 1000 m. This is the point at which the limit of the boundary layer established in the model was located. Fig. 3D shows that a large amount of the particles remained below the height of 1000 m after 8 h.

Fig. 4 shows the simulation of the deposition area and dispersion of PM$_{2.5}$ on February 27, 2015, starting at 09:08 h (UTC-03). In the simulation shown in Fig. 4A and B, the averages of the deposition in the region showed that the plume moved to the northwest two hours after the start of the simulation and exceeded the circle.
of 30 km distance from the emission source, reaching nearby municipalities such as Jandaíra - RN and the surrounding communities. The maximum deposition in the simulated period was 150 μg/m² and occurred near the emission source within the circle of 10 km to the northwest, mainly affecting the community of Amarelão itself. Fig. 4C represents the same analysis of the deposition, but was calculated for four hours after the simulation and exceeded the circle of 40 km with a maximum deposition of 160 μg/m². Fig. 4D shows average PM concentrations in a layer from 0 m to 1500 m that was integrated into the period. The maximum PM2.5 concentration after 2 h of simulation was 13 μg/m³, whereas the point of greatest concentration was located inside the circle at a 10 km distance from the source.

3.4. Biological monitoring

Demographic characteristics of the study population are summarized in Table 2. The control group consisted of 54 men and 62 women, between 18 years and 77 years (mean age: 25.6 ± 8.8). The cashew nut roasting workers included 44 men and 33 women between 18 years and 67 years of age (mean age: 29.9 ± 11.9). No significant difference was observed between the exposed and control groups regarding gender (p = 0.15), age (p = 0.06) and family history of cancer (p = 0.09). However, significant difference was observed in alcohol consumption (p = 0.03), abortion rate (p = 0.0003) and congenital anomalies (p = 0.0005).

Fig. 5A presents the 1-OHP levels according to exposure status, illustrating that the levels among cashew nut workers were higher (p < 0.0001) than those of the control group. In the control group, an average 1-OHP level of 0.031 μmol/mol creatinine was observed with a range of 0.013–0.218. However, the observed average in the cashew nut workers was 0.108 μmol/mol creatinine, with a range of 0.012–0.748.

The average frequency for all the analyzed BMCyt biomarkers was significantly higher in the exposed group than in the control group (p < 0.0001). The MN (Fig. 5B) was the BMCyt biomarker that showed the highest frequency increase compared to the control group (5.0-fold), followed by karyolytic cells (2.2-fold), binucleated cells (2-fold), pyknotic cells (1.8-fold), karyorrhexis (1.6-fold), condensed chromatin (1.6-fold), nuclear buds (1.4-fold) and basal...
Fig. 4. Analysis of the deposition area (μg/m²) and dispersion (μg/m³) of PM$_{2.5}$ on February 27, 2015, simulated with the HYSPLIT-4.0 model in the cashew nuts roasting area, using reanalysis data of the National Center for Environmental Prediction/National Center for Atmospheric Research (NCEP/NCAR). (A) Deposition area after 2 h of simulation using an orographic map of the region. (B) Deposition area after 2 h of simulation, showing the distance traveled by the plume. (C) Deposition area after 4 h, and (D) Dispersion of the particles after 2 h of simulation.
cells (1.2-fold; Table 3). Furthermore, a positive correlation (p < 0.01) among all BMCyt biomarkers was observed (Table S1).

The correlation analysis was positively associated with roasting working time (Fig. 5D) to emphasize the relation between MN frequency and cashew nut roasting activity. Table 4 shows the effect of host factors (gender and age) and lifestyle (alcohol consumption) on the MN frequency according to exposure status. When compared within each group, no significant difference according to the gender and alcohol consumption was observed. In contrast, the effect of age-class was marked by an increase in MN frequency (p < 0.05) in individuals older than 30 years only for the control group. The MN frequencies were statistically (Spearman’s ρ = 0.09) in individuals older than 30 years (p < 0.05) in individuals older than 30 years for all analyses performed for the different categorical independent variables between both groups.

In addition, a significant and positive correlation (Spearman’s ρ = 0.65; p < 0.0001) was observed between the biomarkers of exposure (1-OHP) and effect (micronuclei; Fig. 5C). This correlation was observed for both frequencies obtained from the exposed group (Spearman’s ρ = 0.72; p < 0.0001) and those recorded in the control group (Spearman’s r = 0.33; p = 0.02; Fig. S4).

4. Discussion

This study evaluated the exposure of artisanal cashew nut roasting workers to PM and PAH. It was found that these workers experienced high exposure levels of PM$_{2.5}$, associated with increased urinary levels of 1-OHP. This high exposure condition seems to have reflected on the genotoxic and cytotoxic outcomes observed; i.e., the increase of BMCyt biomarkers and the MN were positively associated with 1-OHP levels.

In October 2013, the International Agency for Research on Cancer (IARC) classified outdoor air pollution as carcinogenic to humans (Group 1) (Loomis et al., 2013; IARC, 2013a). Recent estimates suggested that exposure to PM$_{2.5}$ contributed to 3.2 million premature deaths worldwide in 2010, mainly due to cardiovascular disease, and 223,000 deaths from lung cancer (Lim et al., 2012; IARC, 2013b).

The results obtained from PM$_{10}$ in the Amarelêo community indicated that workers were exposed to concentrations that exceeded the level defined as “a state of emergency” of 500 μg/m$^3$ established by the National Council for the Environment of Outdoor PM$_{10}$ (CONAMA, 1990). Brazil does not have workplace exposure limits for respirable particles, but when compared to the threshold limit value (TLV) established by the American Conference of Governmental Industrial Hygienists (3 mg/m$^3$), and permissible exposure limit (PEL) by the Occupational Health and Safety Administration (5 mg/m$^3$), some measurements were obtained in the range that allowed for acquiring hazardous health effects (OSHA, 2004; ACGIH, 2005).

TSP concentrations measured by the real-time aerosol monitor were slightly higher than measured using the gravimetric sampling method. However, direct-reading instruments have several limitations. Unlike the gravimetric sampling method, the aerosol is not collected into a filter; this fact eliminated the possibility of chemical analyses on the aerosol sample (Kim et al., 2004; Mohar et al., 2013). In addition, the response of this instrument could be affected by the different properties of aerosol, as the principle of light scattering depends on the particle size distribution, refractive index, and particle light absorption, thus causing an overestimation in the measurements (Hinds, 1999; Kim et al., 2004; Kingdom et al., 2006).

Single particle analyses contribute to understanding optical properties, atmospheric heterogeneous reactions, hygroscopic behavior and health evaluation of aerosol particles (Li et al., 2016). Techniques of scanning electron microscopy (SEM) coupled with energy-dispersive x-ray spectroscopy (EDS) have been used in several studies to better characterize airborne particle matter (Braniš and Safranek, 2011; Chung et al., 2012; Wagner et al., 2013; Wilkinson et al., 2013), mainly because they simultaneously provide information about the size, morphology and chemical composition of the particles. However, SEM is insufficient for
observing ultrafine particles having diameters less than 0.1 μm (Li et al., 2016). It is important to emphasize that the sizes obtained through SEM analyses corresponded to physical sizes rather than aerodynamic diameters, which are more associated with the classification of PM10 and are determined using multiple-stage impactors (Huertas et al., 2012).

Both PM2.5 (De Oliveira Galvão et al., 2014) and TSP sampled in the present study showed K, Cl, S, Ca and Fe as the most abundant elements found in the cashew nut roasting process. These elements are typical from a biomass burning source (Lara et al., 2005; Reid et al., 2005). According the classification criteria of single aerosol particles defined by Li et al. (2016), K-rich particles are associated with PM10.

Table 3
The buccal micronucleus cytome assay (BMCyt) endpoints evaluated in the cashew nut workers and control group.

<table>
<thead>
<tr>
<th>BMCyt biomarker</th>
<th>Group</th>
<th>% ±S.D.</th>
<th>Median</th>
<th>Q25-Q75</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of micronuclei</td>
<td>Control</td>
<td>1.03 ± 0.83</td>
<td>1</td>
<td>0.5–1.5</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>5.23 ± 1.41*</td>
<td>5</td>
<td>4–5.5</td>
<td></td>
</tr>
<tr>
<td>Nuclear buds</td>
<td>Control</td>
<td>1.29 ± 1.08</td>
<td>1</td>
<td>1–1.5</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>1.77 ± 1.12*</td>
<td>1.5</td>
<td>1–2</td>
<td></td>
</tr>
<tr>
<td>Binucleated</td>
<td>Control</td>
<td>4.94 ± 2.20</td>
<td>5</td>
<td>3–6</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>10.04 ± 2.73*</td>
<td>10</td>
<td>8–11.5</td>
<td></td>
</tr>
<tr>
<td>Pyknosis</td>
<td>Control</td>
<td>3.45 ± 1.85</td>
<td>3</td>
<td>2–5</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>6.23 ± 2.61*</td>
<td>6</td>
<td>5–8</td>
<td></td>
</tr>
<tr>
<td>Karyolysis</td>
<td>Control</td>
<td>31.03 ± 20.13</td>
<td>25</td>
<td>18–38.75</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>69.10 ± 20.64*</td>
<td>66</td>
<td>54–84</td>
<td></td>
</tr>
<tr>
<td>Karyorrhexis</td>
<td>Control</td>
<td>4.30 ± 2.43</td>
<td>4</td>
<td>3–5.75</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>6.84 ± 2.94*</td>
<td>6</td>
<td>5–8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Condensed chromatin</td>
<td>Control</td>
<td>4.61 ± 1.82</td>
<td>4.5</td>
<td>3–6</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>7.54 ± 3.27*</td>
<td>7</td>
<td>5–9</td>
<td></td>
</tr>
<tr>
<td>Basal cells</td>
<td>Control</td>
<td>18.68 ± 10.10</td>
<td>17</td>
<td>11–23</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>22.04 ± 5.62*</td>
<td>21</td>
<td>18–26</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference compared to the control group (Mann-Whitney test).
with the biomass burning process, and the major elements contained are K, S, Cl, N, and C. The average particle size distribution analyzed by SEM was 7.3 ± 4.8 μm with the prevalence of smaller particles, although this figure may have been underestimated since the smaller particles tend to penetrate the mesh of the quartz fiber filter. This observation corroborated with the results for particulate measurements by real-time aerosol monitors, which found a prevalence of PM$_{2.5}$ in the Amarelão Community.

Guyon et al. (2003) showed the relative importance of fine aerosol particles on the number, volume, and mass size distributions in the Amazon tropical forest during biomass burning events. In the case of cashew nut roasting, the proximity to the source also contributed to the prevalence of fine particles in the total PM mass. In addition, the control area (UFRN/CB) is located in a city surrounded by dunes, and these regions are characterized by coarse aerosol particles that contribute to the higher concentration (mass) of PM. Andreae et al. (2015) verified the influence of coarse particles from Saharan dust in the Amazon region. The aerosol volume distribution indicated a large enhancement of coarse particles, which increased the integrated particle volume concentration by almost one order of magnitude.

A specific particle type was identified from biomass-burning emissions; it was given the name ‘tar ball’ (Pösfai and Buseck, 2010). These particles are carbonaceous spherules formed by organic substances that occur as a result of biomass and biofuel burning. The particles found in Fig. 2B were similar to the tar ball described and characterized by Pösfai et al. (2003, 2004) in samples of young smoke from a smoldering and flaming fire. In general, this spherical morphology suggested that the particles could have been formed during high-temperature conditions and were associated with fossil fuels and biomass burning (Chung et al., 2012; Li et al., 2016). According to Li et al. (2016) aerosol types such as graphic spherules and spherical organic particles showed mixing properties such as S-rich and K-rich particles and were associated with fossil fuels and biomass burning processes. The morphology shown in Fig. 2E (smooth-surfaced and cubic forms) is similar to the corn cob flame soot particles (Li et al., 2016), with the elements K and Cl predominating in both cases.

HYPLIT model simulations were both consistent with the reanalysis data, as well as with the surface data from INMET weather stations in the periods where the direction of the pollutant dispersion emitted by cashew nut burning was analyzed. The city of João Câmara - RN, is located close to latitude 6° South. This implies that the wind patterns were usually predominated by southeast trade winds from the dragging effects of the Earth’s rotation and that there is a predominance of wind towards the northwest (Motta, 2004). The PM$_{2.5}$ was predominantly dispersed in the northwest direction, being an important factor despite the fact that dispersion and deposition of PM$_{2.5}$ can impact the surrounding regions. This fact was underestimated, since the emission of PM by artisanal cashew nut roasting lasts for approximately 10 h every day (De Oliveira Galvão et al., 2014).

Measurements of biological markers of exposure (i.e., 1-OHP) and effect (i.e., BMCyt endpoints) can improve investigation of the health risk caused by air pollution exposure by facilitating improved exposure assessment and increasing understanding of action mechanisms, thereby providing biological reliability (Demetriou et al., 2012).

The results of 1-OHP obtained in this study is in accordance with previous findings of diesel-derived occupational exposure assessment in bus garages and waste-collection workers, where the control level was 0.05 ± 0.09 μmol/mol creatinine and 0.12 ± 0.16 and 0.15 ± 0.19 μmol/mol creatinine for the exposed group (Kuusimäki et al., 2004). In a recent study, Yuan et al. (2015) investigated the influence of PAH exposure among 781 adults living near a large petrochemical complex in Taiwan. They found an average urinary 1-OHP concentration of 0.15 ± 0.29 μmol/mol- creatinine, similar to those observed in cashew nut roasting workers in this study (0.108 ± 0.16 μmol/mol- creatinine).

Although the 1-OHP is widely used as a marker of PAH exposure, it is not always reliable because its concentration may be affected by both environmental and behavioral factors, as well as genetic polymorphisms in PAH metabolizing enzymes (Ciarrocca et al., 2014).

A positive correlation between the 1-OHP levels and MN frequency in exfoliated buccal mucosa cells was observed (r = 0.65; p < 0.0001; Fig. 5C). Leng et al. (2004) investigated the effect of PAH among coke-oven workers and verified a similar correlation but for MN frequency in peripheral blood lymphocytes (r = 0.383; p < 0.01). Duan et al. (2009) also evaluated coke-oven workers’ exposure using the cytokinesis-block micronucleus (CBMN) cyto- assay and found a correlation between 1-OHP levels, nucleoplasmic bridges and nuclear buds (r = 0.741; p < 0.001 and r = 0.64; p < 0.001, respectively). However, when compared with 1-OHP, the MN was a more sensitive biomarker to cashew nut roasting elements (Fig. 5). 1-OHP is a specific biomarker for PAH exposure since it is the product of pyrene metabolism, whereas MN is a wider biomarker of clastogenic and/or aneugenic effects. This finding explains the difference in sensibility observed among these biomarkers (Fig. 5A and 5B).
In the present study, it was observed that the MN frequency for the control group was 1.03%. This result is in accordance with Bonassi et al. (2011) who established values for a suitable interval (0.32–1.70%) that can be considered as the natural occurrence of MN in non-exposed populations. Furthermore, the rate of MN resulting from the biomass burning of cashew nuts was higher than that obtained by several studies that used a BMCyt assay to assess workers occupationally exposed to pesticides in soybean farm workers (Benedetti et al., 2013), agricultural workers (Carbajal-Lopez et al., 2016), wood dust (Wultsch et al., 2015), and cotton weavers (Khan et al., 2015), coal miners (Rohr et al., 2013) and foundry workers (Singaravelu and Sellappa, 2015).

Our group used a plant model (Tradescantia pallida) to assess the genotoxicity of cashew nut roasting, and we also observed a notably high MN rate compared to other air pollutant sources (De Oliveira Galvão et al., 2014). The high frequency of MN measured in T. pallida, as well as the confirmation in the exfoliated buccal mucosa human cells, strengthened the evidence for a high genotoxic potential of these pollutants.

A meta-analysis study conducted by Ceppi et al. (2010) estimated that age (98.4%), smoking habit (90.5%), and gender (85.7%) were the most common confounders to MN frequency in BMCyt. An international collaborative study performed by Bonassi et al. (2011) indicated age as an important predictor of MN frequency. Sisenando et al. (2012) analyzed children exposed to biomass burning in the Brazilian Amazon and also found constantly increasing MN frequency according to age. Despite no differences being observed for age and gender in comparing the control and exposed groups (Table 2), when the data were stratified into these categories, the same increase of MN frequency was found in individuals older than 30 years, but only for the control group (Table 4).

Correlation analysis between observed BMCyt endpoints validated the use of other biomarkers, but MN was the most effective biomarker to assess cashew nut roasting pollutants. The application of additional biomarkers in the cytome approach can expand the scope and utility of the BMCyt assay by measuring a wider diversity of cellular pathologies (Bolognesi et al., 2015) and distinguishing between genotoxic, cytostatic and cytotoxic effects (Bolognesi et al., 2013).

For all cytotoxic biomarkers (pyknosis, karyolysis, karyorrhexis and condensed chromatin) a significant increase (p < 0.0001) was observed when compared to the control group (Table 3). In addition, the analysis among BMCyt endpoints showed the highest positive correlation between the condensed chromatin and karyorrhexis cells (r = 0.79, p < 0.01, Table S1). Although still not conclusive, chromatin condensation may represent an early stage of apoptosis, while karyorrhexis is associated with a later stage (Thomas et al., 2009; Bolognesi et al., 2013).

When comparing the cytotoxic biomarkers, karyolysis was the most sensitive (increased 2.5-fold compared with the control, Table 3). Karyolysis represents the stage when disintegration of the nucleus is complete and occurs in the later stages of necrosis and apoptosis (Thomas et al., 2009; Bolognesi et al., 2013).

Furthermore, an increase in the frequency of abortions (p < 0.0003) and congenital anomalies (p < 0.0005) were observed in the exposed group compared to the control group (Table 2). PM can cross the placental barrier and trigger its effects on the fetus. Several human studies have associated the exposure to PM and PAHs with various negative effects on pregnancy and birth development, such as: delayed intrauterine growth, decrease of head circumference, birth size and weight, premature birth and missed abortion (Perera et al., 2005; Choi et al., 2008; Wu et al., 2010; Lamichhane et al., 2015; Defranco et al., 2016).

The ability to produce high-quality scientific research that addresses the assessment of risks between air pollution exposure and human biological effects, and the capacity of authorities to understand and react to these risks are basic requirements to solve the conflict between economic development, social conflicts and the preservation of human health (Fajersztajn et al., 2013). In this sense, one of the major contributions of the data presented in this study is the fact that it can be an important source of information for establishing a future guideline for environmental policies to promote air pollutant control and to communicate the risk as well as the need that urgent action is required from public officials to propose occupational improvements such as the organization of associations, providing mini-factories with exhaust systems that eliminate air pollutants and the use of gloves and masks during the cashew nut roasting process.

5. Conclusions

The results of this study suggest that artisanal cashew nut roasting is a hazardous occupation, where exposure causes harmful effects on workers' health. The physico-chemical and simulation analyses of PM showed typical elements from biomass burning with the potential to reach neighboring regions of the emission source. PAH exposure was verified by increases in urinary 1-OHP levels and the genotoxic/cytotoxic potential was confirmed by the increase in BMCyt biomarkers in cashew nut workers. The micro-nucleus was the most sensitive BMCyt biomarker analyzed and positively correlated with 1-OHP levels.

Therefore, using exposure and effect biomarkers was efficient in assessing the occupational risk associated with artisanal cashew nut roasting. The high rates of fine PM are considered a potential contributor to this effect and the use of portable real-time aerosol monitors in areas of difficult access are an alternative to an initial screening of PM measurements.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.10.024.

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