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Cashew nut roasting: Chemical characterization of particulate matter and genotocixity analysis

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ABSTRACT

Background: Particulate matter (PM) is potentially harmful to health and related to genotoxic events, an increase in the number of hospitalizations and mortality from respiratory and cardiovascular diseases. The present study conducted the first characterization of elemental composition and polycyclic aromatic hydrocarbon (PAH) analysis of PM, as well as the biomonitoring of genotoxic activity associated to artisanal cashew nut roasting, an important economic and social activity worldwide.
Methods: The levels of $PM_{2.5}$ and black carbon were also measured by gravimetric analysis and light reflectance. The elemental composition was determined using X-ray fluorescence spectrometry and PAH analysis was carried out by gas chromatography–mass spectrometry. Genotoxic activity was measured by the *Tradescantia pallida* micronucleus bioassay (Trad-MCN). Other biomarkers of DNA damage, such as nucleoplasmic bridges and nuclear fragments, were also quantified.
Results: The mean amount of $PM_{2.5}$ accumulated in the filters (January 2124.2 $\mu g/m^3$; May 1022.2 $\mu g/m^3$; September 1291.9 $\mu g/m^3$), black carbon (January 363.6 $\mu g/m^3$; May 70 $\mu g/m^3$; September 69.4 $\mu g/m^3$) and concentrations of Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br and Pb were significantly higher than the non-exposed area. Biomass burning tracers K, Cl, and S were the major inorganic compounds found. Benzo[k]fluoranthene, indene[1,2,3-c,d]pyrene, benzo[ghi]perylene, phenanthrene and benzo[b]fluoranthene were the most abundant PAHs. Mean benzo[a]pyrene-equivalent carcinogenic power values showed a significant cancer risk. The Trad-MCN bioassay revealed an increase in micronucleus frequency, 2–7 times higher than the negative control and significantly higher in all the months analyzed, possibly related to the mutagenic PAHs found.
Conclusions: This study demonstrated that artisanal cashew nut roasting is a serious occupational problem, with harmful effects on workers' health. Those involved in this activity are exposed to higher $PM_{2.5}$ concentrations and to 12 PAHs considered potentially mutagenic and/or carcinogenic. The Trad-MCN with *T. pallida* was sensitive and efficient in evaluating the genotoxicity of the components and other nuclear alterations may be used as effective biomarkers of DNA damage.

1. Introduction

About 31 countries worldwide produced 4.20 million metric tons of cashew nuts in 2011. The major cashew nut producing countries and their production figures are Vietnam (1.27 million tons), Nigeria (0.81 million tons), India (0.67 million tons), Ivory Coast (0.45 million tons), Brazil (0.23 million tons) and the Philippines (0.13 million tons). In 2010, major exporters of shelled cashew nuts included Vietnam, India and Brazil and the main importing countries were the USA (119.11 metric tons), the Netherlands (41.27 metric tons), Germany (25.44 metric tons) and China (23.44 metric tons) (FAO, 2013).

The semi-arid region of Brazil is characterized by an annual rainy season and an extremely dry period. Cashew nut roasting is a socially accepted and financially viable alternative, since, in addition to being a year-round activity, it produces an easily commercialized product. However, the lack of assistance provided to workers,

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the informality of the activity and the lack of knowledge on the part of society in general about the conditions under which cashew nut roasting takes place, hamper control of the potential harmful effects associated to this type of enterprise.

One of the main issues in the cashew fruit productive chain are the conditions under which cashew roasting takes places to obtain the kernel. The nuts are oven-roasted, where the shell itself acts as the fuel. The fuel generated from cashew nut roasting releases cashew nut shell liquid, a highly flammable caustic phenolic oil that releases compounds into the atmosphere (Agila and Barringer, 2011; Chandrasekara and Shahidi, 2011). The smoke generated during cashew nut roasting is inhaled daily by family groups that take part in the activity for periods that may exceed 10 h/day. The roasting process generally occurs between 2:00 AM and 12:00 h PM.

Biomass burning releases several air pollutants, especially particulate matter (PM), a complex mixture including inorganic and organic compounds. Fine particulates ($PM_{2.5}$) are potentially harmful to health and may affect the lower airways, reaching the pulmonary alveoli. These particles are related to genotoxic events, an increase in the number of hospitalizations and ambulatory visits, and mortality from cardiovascular and respiratory diseases (Alves et al., 2011; André et al., 2012; De Oliveira et al., 2012; Da Silva et al., 2012). The organic fraction of PM has recently caused significant environmental concern due to the high mutagenic and carcinogenic potential displayed by its components (Vasconcellos et al., 2010). Data on the composition of PM smoke are important in order to understand the organic component contributions of biomass burning emissions to atmospheric chemistry, in addition to complementing existing research on the characterization of direct (natural) organic emissions from biomass sources (Simoneit, 2002).

Plant bioassays are best suited to assessing the effects of air pollution and are generally highly sensitive in detecting the genotoxic effects (Villarini et al., 2009; Alves et al., 2011, 2014; Sisenando et al., 2011). The Tradescantia micronucleus bioassay (Trad-MCN) has been extensively used in genotoxicity studies, due to its high sensitivity to such compounds (Mišík et al., 2011). However, in tropical countries Tradescantia clones, mainly BNL-4430 and KU-20, do not grow well in the high temperature, humidity and rainfall conditions typically found in these regions, often exhibiting inhibited growth and flowering (Suyama et al., 2002). An appropriate alternative to the environmental conditions found in tropical countries is the use of *Tradescantia pallida*. Several studies have demonstrated its sensitivity to the genotoxic compounds from air pollutants *in situ* (Batalha et al., 1999; Guimarães et al., 2000; Carreras et al., 2006; Prajapati and Tripathi, 2008; Mariani et al., 2009; Meireles et al., 2009; Savóia et al., 2009; Sisenando et al., 2011; Pereira et al., 2013) and *ex situ* (Carvalho-Oliveira et al., 2005; Alves et al., 2011, 2014; Carreras et al., 2013).

Owing to this exposure, the aim of the study was to assess the concentration, elemental composition and polycyclic aromatic hydrocarbon (PAH) analysis of PM, as well as the genotoxic potential associated to artisanal cashew nut roasting during the dry, rainy and intermediate seasons in a semiarid region of Brazil.

2. Materials and methods

2.1. Study area

Two sites were chosen as test areas: (1) The community of Amarelão, located on the rural perimeter of the municipality of João Câmara, Brazil (05°30'51.81"S; 35°54'17.13"O), site of the cashew nut roasting. (2) Santa Luzia farm (05°33'6.72"S; 35°46'10.75"O), situated 13 km from the roasting location and exhibiting the same environmental conditions, but without the influence of cashew nut processing. The two sites are not directly affected by vehicular emissions or industrial pollutants. Fig. 1 shows the location of the sampling site, which is downwind from the two areas.

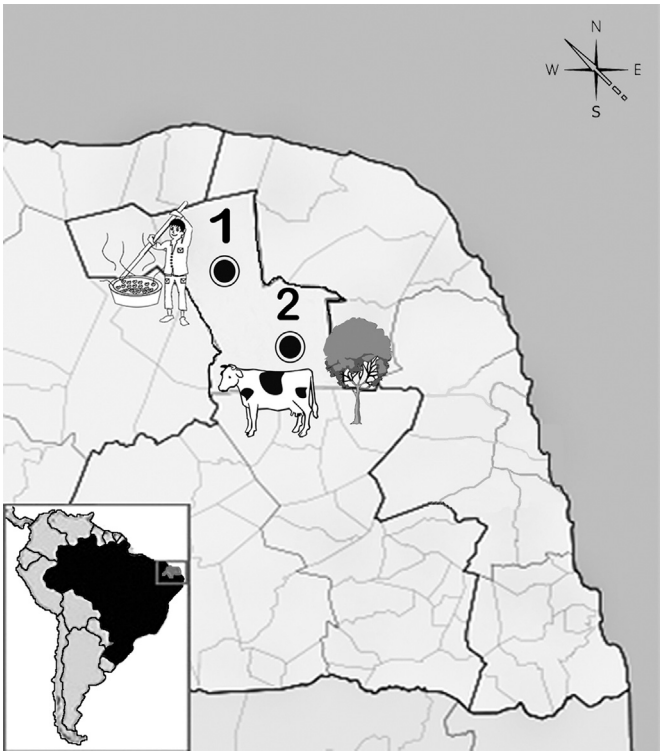

Fig. 1. Location of the artisanal cashew nuts roasted. Exposure sites of *Tradescantia pallida* and the $PM_{2.5}$ collection sites. Site 1 (Amarelão Community) and Site 2 (Santa Luzia farm). The arrow indicates the prevailing wind direction.

2.2. Fine particulate matter sampling

Fine particulate matter ($PM_{2.5}$) was sampled in January (dry season), May (rainy season) and September (intermediate season) 2009, using a portable sampler (manufactured by the Harvard School of Public Health) equipped with a $PM_{2.5}$ probe and at a flow rate of 1.8 lpm, with a polycarbonate membrane (diameter of 37 mm, pore 0.8 μm, Millipore-USA). Each campaign sampled 10 filters per site, obtaining a total of 60 filters.

Each filter was submitted to gravimetric analysis using a Mettler MT5 microbalance (Mettler-Toledo, Greifensee, Switzerland), with a minimum resolution of 1 μg, to estimate average daily mass concentration. Black Carbon (BC) concentration was estimated by optical reflectance using a Smoke Stain Reflectometer (Model 43D, Diffusion Systems LTD, London, UK) (Reid et al., 1998).

2.3. Total suspended particle sampling

Total suspended particles (TSP) were sampled in March 2013 only at the Amarelão site, using a Handi-vol sampler (Energética, Brazil) operating at a flow rate of 230 lpm, with a quartz fiber filter (EQTZ diameter of 110 mm, Energética, Brazil). Each filter was previously conditioned in an oven for 8 h at 800 °C, to avoid contamination during PAH laboratory analysis.

2.4. Elemental composition of fine particulate matter

The elemental composition of $PM_{2.5}$ was determined by energy dispersive X-ray fluorescence spectrometry analysis (ED-XRF) using an EDX-700HS model (Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). A low power Rh-target tube, with voltage of 5–50 kV and current from 1 to 1000 μA, was used in the analyses. The characteristic X-ray radiation was detected by a Si (Li) detector. Analysis was performed in a vacuum atmosphere on a 10 mm diameter surface, for the elements Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br and Pb.

2.5. Organic compounds of total suspended particles

Organic compounds were extracted from the filters by ultrasonic bath (3 cycles of 20 min), with 80 mL dichloromethane (DCM) as solvent for each cycle. Sample extracts were concentrated to 5 mL by a rotary evaporator and then under gentle N_2 flux. The different fractions were obtained using a silica gel (3.4 g) and alumina (1.8 g) column. The column was pre-washed with 20 mL of *n*-hexane. The first fraction containing the *n*-alkanes was eluted from the silica gel column with 40 ml

of *n*-hexane. PAHs were eluted with a mixture of 50 mL of *n*-hexane and 50 mL of DCM. This study focused on PAH fractions. Quantitative and qualitative analyses were carried out by gas chromatography–mass spectrometry (Agilent 7820A+5975 MSD). A 1 μL sample was injected in splitless mode (Vasconcellos et al., 2010). Benzo(a)pyrene-equivalent carcinogenic power (BaPE) was calculated using the formula provided by Yassaa et al. (2001), as follows:

$$BaPE = BaA \times 0.06 + B(b+k)F \times 0.07 + BaP + DBA \times 0.06 + InP \times 0.08 \quad (1)$$

2.6. Tradescantia micronucleus test (Trad-MCN)

Tradescantia pallida was cultivated in a greenhouse under controlled temperature and humidity (30 ± 2 °C, 70%). Seed dispersion and plant exposure were conducted in 30 plastic flowerpots (50 cm × 17 cm), using a mixture of standard soil composed of two parts plant soil, one part Eucatex® Plantmax HT substrate, one part fine vermiculite and one part worm humus (2:1:1:1 v/v). The plants were watered daily with distilled water, in the dispersion period (under controlled conditions), acclimation and exposure periods. Before the first collection, the plants underwent a one-month adaptation period at the two test sites. For each test site 30 young inflorescences were collected monthly and fixed in 45% acetic acid solution and 70% ethyl alcohol (1:3 v/v) for 48 h, and then preserved in 100% ethyl alcohol, according to an adaptation of the protocol established by Ma et al. (1994). The inflorescence samplings were done at each site from August 2008 to September 2009. All samplings occurred in months with intense cashew nut roasting activity and campaigns conducted in January, May and September 2009 occurred simultaneously to the collection and analysis of $PM_{2.5}$. The rainfall data for the biomonitoring period were obtained from the Agricultural Research Company of Rio Grande do Norte (EMPARN).

For cytological analysis the cells were stained with 2% carmine acetate and heated to approximately 80 °C for better stain fixation. A total of 10 slides were analyzed, equivalent to 3000 tetrads per site per month, and three chromosomal biomarkers of genomic instability were quantified: micronuclei (MN), nucleoplasmic bridges (NPB) and nuclear fragments (NF) (Fig. 2). The count was conducted under optical microscope (Olympus CX31RBSFA) at 400X magnification.

2.7. Statistical analysis

The Anderson–Darling test was applied to determine whether the results obtained exhibited normal distribution, with $\alpha = 0.05$ (normal when $p \geq \alpha$). The means of all the results were calculated and, for those that did not show normal distribution, a calculation of the median was performed. The dispersion of the results was verified using standard deviation (SD) and interquartile distance (IQR) for those that did not exhibit normal distribution.

To determine if there were significant differences between the results ($p \leq 0.05$), data that showed normal distribution were submitted to ANOVA and those that did not to the Kruskal–Wallis test. The data obtained for MN, NPB and NF were submitted to Dunnett's multiple comparison test to analyze significant differences. The correlation between the biomarkers of DNA damage and rainfall was defined on the basis of Pearson's correlation matrices. Statistical analyses were performed using GraphPad Prism 5, Minitab 15 software, and Microsoft Excel®, 2007.

3. Results

3.1. Analysis of the $PM_{2.5}$ accumulated in the polycarbonate filters

The results obtained in January, May and September 2009 for concentrations of $PM_{2.5}$, BC and elemental composition are shown in Table 1. The results of $PM_{2.5}$ and BC were significantly higher at the Amarelão community than at the farm. For the former, the $PM_{2.5}$ results differed significantly among the three collection campaigns, with the largest concentration found in January (2124.2 μg/m^3), followed by September (1291.9 μg/m^3) and May (1022.2 μg/m^3). BC showed similar results in May (70.0 μg/m^3) and September (69.4 μg/m^3), significantly lower than those of January (363.6 μg/m^3). The results obtained for $PM_{2.5}$ and BC at the farm during all the campaigns were similar, showing no significant differences for these two variables (Table 1).

For all the campaigns in which they were present, the elements Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br and Pb exhibited higher concentrations at the Amarelão community. Higher concentrations of Cl > K > S > Fe were also identified there in January, K > Si > S > Cl > Cu in May and K > Si > Cl > S in September (Table 1).

3.2. PAH analysis

The individual PAH concentrations of the samples collected at the Amarelão site are shown in Fig. 3. In this study, 17 PAH compounds, listed as priority by the United States Environmental Protection Agency (US-EPA, 2010), were identified and quantified, as follows: acenaphthalene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), retene (Ret), benz[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[e]pyrene (BeP), benzo[a]pyrene (BaP), indene[1,2,3-c,d]

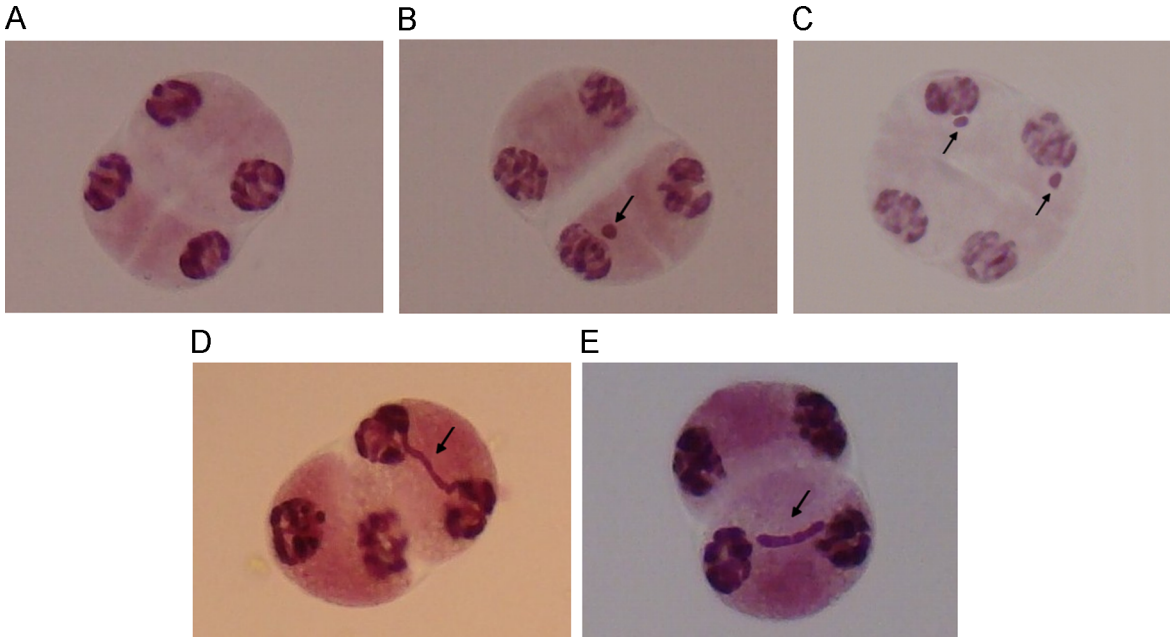

Fig. 2. Tetrads of *Tradescantia pallida* stained with 2% carmine acetate and viewed under optical microscope, 400X. (A) Morphology tetrads without nuclear alterations, (B) the presence of tetrad with one micronucleus (MN), (C) tetrad with two micronuclei, (D) Tetrad with a nucleoplasmic bridges (NPB), (E) Tetrad with the presence of a nuclear fragment (FN), arrows indicate MN, PNP and FN.

Table 1
Measurements of particulate matter fine ($PM_{2.5}$), black carbon (BC) and elemental composition in January, May and September 2009.

Element	January-2009		May-2009		September-2009	
	Site 1	**Site 2**	**Site 1**	**Site 2**	**Site 1**	**Site 2**
$PM_{2.5}$						
Median-IQR	187.750–2017.87	24.15–3.65	486–1291	20.72–15.13	782.4–982.32	23.18–14.84
Mean ± SD	2124.2* ± 3635.5	26.23 ± 4.89	1022.23* ± 907.081	21.112 ± 12.091	1291.95* ± 1274.01	29.442 ± 23.760
BC						
Median-IQR	11.318–339.67	0.436–0.135	46.9–35.33	0.185–0.11	46.8–42.34	0.639–0.287
Mean ± SD	363.623* ± 656.81	0.449 ± 0.135	70.068* ± 75.845	0.194 ± 0.092	69.432* ± 69.054	0.680 ± 0.199
Al						
Median-IQR	0.095–0.262	0.000–0.074	0.000–0.000	0.085–0.059	0.000–0.000	0.000–0.011
Mean ± SD	0.119 ± 0.130	0.025 ± 0.040	0.000 ± 0.000	0.098 ± 0.031	0.000 ± 0.000	0.004 ± 0.007
Si						
Median-IQR	0.105–0.693	0.084–0.039	2.308–19.890	0.266–0.227	4.040–22.591	0.035–0.435
Mean ± SD	0.280 ± 0.380	0.072 ± 0.020	8.339 ± 11.233	0.275 ± 0.114	9.259 ± 12.166	0.180 ± 0.058
P						
Median-IQR	0.005–0.015	0.005–0.006	0.000–0.000	0.000–0.000	0.000–0.122	0.000–0.010
Mean ± SD	0.007 ± 0.007	0.004 ± 0.003	0.000 ± 0.000	0.000 ± 0.000	0.041 ± 0.070	0.003 ± 0.005
S						
Median-IQR	0.545–2.077	0.104–0.070	0.543–0.446	0.073–0.044	0.439–0.357	0.180–0.094
Mean ± SD	1.023* ± 1.120	0.121 ± 0.04	0.546* ± 0.223	0.076 ± 0.022	0.456* ± 0.179	0.199 ± 0.088
Cl						
Median-IQR	2.123–2.971	0.120–0.707	0.162–0.686	0.126–0.187	0.310–1.168	0.026–0.009
Mean ± SD	1.786* ± 1.510	0.302 ± 0.390	0.283* ± 0.223	0.104 ± 0.022	0.608* ± 0.639	0.023 ± 0.005
K						
Median-IQR	1.031*–2.257	0.136–0.243	1.061–3.475	0.109–0.244	1.467–3.680	0.173–0.116
Mean ± SD	1.427* ± 1.180	0.200 ± 0.130	2.048* ± 1.936	0.124 ± 0.123	2.349* ± 1.992	0.186 ± 0.059
Ca						
Median-IQR	0.193–0.572	0.088–0.234	0.124–0.296	0.066–0.081	0.109–0.144	0.015–0.007
Mean ± SD	0.310 ± 0.300	0.114 ± 0.120	0.180 ± 0.156	0.056 ± 0.041	0.115 ± 0.072	0.015 ± 0.003
Ti						
Median-IQR	0.000–0.012	0.000–0.001	0.000–0.000	0.000–0.000	0.006–0.009	0.001–0.003
Mean ± SD	0.004 ± 0.010	0.001 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.005 ± 0.005	0.001 ± 0.002
Cr						
Median-IQR	0.000–0.060	0.000–0.000	0.000–0.000	0.000–0.000	0.008–0.097	0.001–0.004
Mean ± SD	0.020 ± 0.034	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.035 ± 0.054	0.001 ± 0.002
Mn						
Median-IQR	0.006–0.018	0.003–0.004	0.000–0.006	0.000–0.002	0.000–0.000	0.000–0.000
Mean ± SD	0.008 ± 0.009	0.003 ± 0.002	0.002 ± 0.003	0.001 ± 0.001	0.000 ± 0.000	0.000 ± 0.000
Fe						
Median-IQR	0.207–0.282	0.007–0.013	0.017–0.046	0.015–0.009	0.004–0.006	0.000–0.005
Mean ± SD	0.163* ± 0.150	0.007 ± 0.010	0.026 ± 0.0242	0.013 ± 0.005	0.003 ± 0.003	0.002 ± 0.003
Ni						
Median–IQR	0.020–0.016	0.003–0.006	0.033–0.027	0.004–0.004	0.022–0.019	0.001–0.001
Mean ± SD	0.016 ± 0.010	0.004 ± 0.000	0.041 ± 0.015	0.005 ± 0.002	0.027 ± 0.001	0.001 ± 0.010
Cu						
Median-IQR	0.045–0.026	0.007–0.037	0.323–0.539	0.023–0.042	0.000–0.000	0.000–0.002
Mean ± SD	0.039 ± 0.010	0.015 ± 0.020	0.325* ± 0.270	0.022 ± 0.021	0.000 ± 0.000	0.001 ± 0.001
Zn						
Median-IQR	0.044–0.041	0.003–0.049	0.220–0.365	0.019–0.030	0.000–0.000	0.000–0.005
Mean ± SD	0.036 ± 0.020	0.019 ± 0.030	0.208* ± 0.183	0.017 ± 0.015	0.000 ± 0.000	0.002 ± 0.003
Se						
Median-IQR	0.004–0.011	0.000–0.001	0.000–0.001	0.000–0.001	0.000–0.005	0.000–0.002
Mean ± SD	0.005 ± 0.010	0.000 ± 0.000	0.000 ± 0.001	0.000 ± 0.001	0.002 ± 0.003	0.001 ± 0.001
Br						
Median-IQR	0.000–0.012	0.000–0.000	0.013–0.038	0.005–0.006	0.000–0.000	0.003–0.003
Mean ± SD	0.004 ± 0.010	0.000 ± 0.000	0.017 ± 0.019	0.005 ± 0.003	0.000 ± 0.000	0.003 ± 0.002
Pb						
Median-IQR	0.009–0.019	0.002–0.002	0.000–0.000	0.000–0.002	0.023–0.098	0.000–0.000
Mean ± SD	0.009 ± 0.000	0.001 ± 0.010	0.000 ± 0.000	0.001 ± 0.001	0.040 ± 0.051	0.000 ± 0.000

The central tendency of the results was presented as median and mean and dispersion as interquartile distance (IQR) and standard deviation (SD). The results are expressed in ($\mu g/m^3$). Site 1 represents the Amarelão community and Site 2, Santa Luzia farm.

* Statistically significant at $p < 0.05$.

pyrene (InP), dibenz[a,h]anthracene (DBA) and benzo[g,h,i]pyrene (BghiP). Among the PAHs identified, Phe, Flt, Pyr, BeP and BghiP are mutagenic, according to the International Agency for Research on Cancer (IARC, 2010). Moreover, BaA, Chr, BbF, BkF, BaP, InP and DBA are also considered to be carcinogenic and mutagenic. BkF > InP > BghiP > Phe > BbF were the most abundant PAHs.

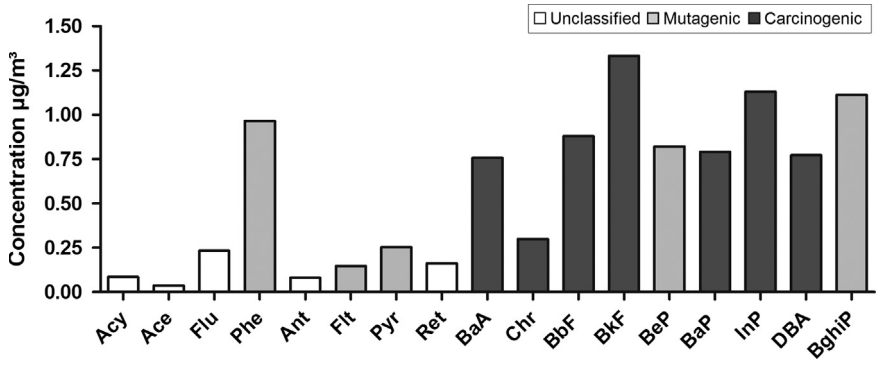

Fig. 3. Results of PAHs identified and quantified in organic matter from the Amarelão community during cashew nut roasting (March/2013).

Table 2
Monthly frequency of micronuclei (MN), nucleoplasmic bridges (NPB), nuclear fragments (NF) and its standard deviation (SD) in inflorescences of *T. pallida* sampled in the Community of Amarelão and Santa Luzia farm, during the period of August 2008–September 2009.

Months/years	MN (%) ± S.D.		NPB (%) ± S.D.		NF (%) ± S.D.		Number of tetrads analyzed	
	Farm	Amarelão	Farm	Amarelão	Farm	Amarelão	Farm	Amarelão
08/2008	1.66 ± 0.81	5.03 ± 1.49***	0.30 ± 0.18	1.03 ± 0.27***	0.10 ± 0.16	0.51 ± 0.27**	3000	3300
09/2008	2.23 ± 1.15	5.63 ± 1.74***	0.50 ± 0.28	1.33 ± 0.49**	0.13 ± 0.23	0.93 ± 0.44***	3000	3300
10/2008	1.33 ± 0.58	5.63 ± 2.76***	0.26 ± 0.21	1.43 ± 0.38***	0.06 ± 0.14	1.03 ± 0.33***	3000	3000
11/2008	3.59 ± 0.75	9.13 ± 1.30***	0.93 ± 0.37	2.06 ± 0.37***	0.96 ± 0.33	1.93 ± 0.49***	3000	3000
12/2008	2.99 ± 0.94	8.20 ± 1.14***	0.86 ± 0.17	1.26 ± 0.64ns	0.36 ± 0.24	0.96 ± 0.42**	3000	3000
01/2009	1.26 ± 0.62	8.46 ± 2.32***	0.13 ± 0.17	–	0.03 ± 0.10	–	3000	3000
02/2009	1.63 ± 0.69	6.76 ± 1.35***	0.40 ± 0.43	1.16 ± 0.39**	0.06 ± 0.14	0.60 ± 0.49**	3000	3000
03/2009	1.69 ± 0.65	5.56 ± 0.54***	0.46 ± 0.52	0.93 ± 0.66ns	0.0 ± 0.0	0.66 ± 0.31	3000	3000
04/2009	1.49 ± 0.42	5.43 ± 1.28***	0.43 ± 0.62	0.90 ± 0.68ns	0.03 ± 0.10	0.20 ± 0.28ns	3000	3000
05/2009	1.46 ± 1.12	5.36 ± 2.02***	–	0.80 ± 0.67	–	0.20 ± 0.23	3000	3000
06/2009	1.86 ± 0.77	5.66 ± 1.06***	0.50 ± 0.36	0.96 ± 0.71ns	0.16 ± 0.23	0.56 ± 0.41**	3000	3000
07/2009	1.93 ± 0.34	5.90 ± 1.42***	0.53 ± 0.42	1.09 ± 0.59*	0.20 ± 0.17	0.85 ± 0.53**	3000	2100
08/2009	1.33 ± 0.47	5.66 ± 1.82***	0.16 ± 0.17	0.56 ± 0.31**	0.0 ± 0.0	0.26 ± 0.37*	3000	3000
09/2009	1.26 ± 0.81	5.76 ± 2.83***	0.16 ± 0.28	0.60 ± 0.46*	0.10 ± 0.16	0.43 ± 0.31**	3000	3000

* Statistically significant at $p < 0.05$.
** Statistically significant at $p < 0.01$.
*** Statistically significant at $p < 0.0001$.
ns Not significant.

The BaPE ranged from 0.3 to 4.8, and the average value was 1.94, higher than the 1.0 considered a significant cancer risk.

3.3. Mutagenic analysis in a plant system

For all the months analyzed during biomonitoring (August 2008 to September 2009), the average frequency of MN in *T. pallida* for the Amarelão community was 2–7 times higher than that of the Santa Luzia farm, ($p < 0.0001$), as shown in Table 2. In the analysis of NPB, except for 12/08, 03/09, 04/09 and 06/09, all the other months analyzed at Amarelão showed a significant increase in the rate of NPB, compared with the farm. For the analysis of NF, except in 07/2008 and 04/2009, the other months analyzed showed a significant increase in the rate of NF (Table 2).

The correlation between $PM_{2.5}$ concentration and mean micronucleus frequency is shown in Fig. 4. In addition, a positive correlation was found between each of the three biomarkers of DNA damage used: MN, NPB and NF (Table 3). This correlation was observed for both the frequencies obtained at the Amarelão community and those recorded on Santa Luzia farm, suggesting that biomarkers other than MN can be used in a Trad-MCN assay. Furthermore, we found a negative correlation between each of the three biomarkers of DNA damage and rainfall during the months of inflorescence collections at the Amarelão site: MN ($r = -0.58$; $p < 0.02$); NPB ($r = -0.76$; $p < .002$) and NF ($r = -0.67$; $p < 0.01$) (Table 3).

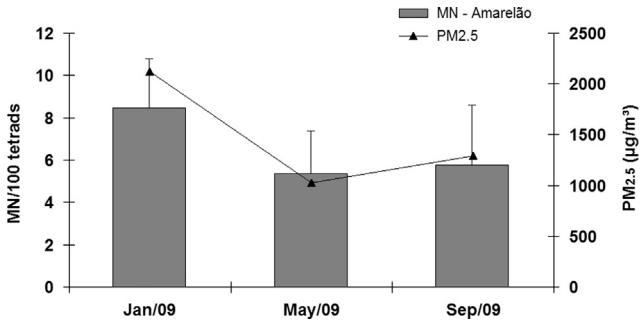

Fig. 4. Relationship between $PM_{2.5}$ and mean MCN frequencies measured in *Tradescantia pallida* during the cashew nut roasting period.

4. Discussion

The results obtained from the PM in the Amarelão community indicate that workers are exposed to concentrations that exceed the level of 500 µg/m^3 (CONAMA, 1990) defined as "state of emergency" by the National Council for the Environment – CONAMA. All measurements of $PM_{2.5}$ obtained exceeded the daily limits established by CONAMA, WHO and the EPA (CONAMA, 1990; WHO, 2005). Thus, the manner in which this activity is currently being carried out is characterized as an occupational hazard, causing serious health risks to the workers involved.

Table 3
Correlation matrix for DNA damage biomarkers of the Trad-MCN assay and rainfall for Community of Amarelão and Santa Luzia farm.

Site	Biomarkers		Rainfall	MN	NF
Amarelão	NPB	*p*	< 0.002	< 0.0003	< 0.0001
		r	−0.76	0.82	0.93
	NF	*p*	< 0.01	< 0.0007	–
		r	−0.67	0.79	–
	MN	*p*	< 0.02	–	–
		r	−0.58	–	–
Farm	NPB	*p*	ns	< 0.0002	< 0.0007
		r	−0.29	0.84	0.79
	NF	*p*	ns	< 0.0001	–
		r	−0.43	0.89	–
	MN	*p*	ns	–	–
		r	−0.41	–	–

ns – not significant.
NPB – nucleoplasmic bridges.
NF – nuclear fragments.
MN – micronuclei.

In a study conducted with women chronically exposed to high levels of indoor air pollution from biomass fuel, average concentrations recorded for PM_{10} and $PM_{2.5}$ were 625 ± 150 μg/m^3 and 312 ± 85 μg/m^3, respectively (Mondal et al., 2010). The authors observed large numbers of MN (CBMN test), elevated oxidative damage (Comet assay), increased ROS and decreased antioxidant enzymes in exposed women. $PM_{2.5}$ levels in the Amarelão community were higher than those found by Mondal et al. (2010).

The biomass burning profile was characterized by high concentrations of K^+, elemental carbon and organic carbon (Simoneit, 2002; Khalil and Ramussen, 2003; Reid et al., 2005) and other typical elements contained in wood smoke (S, Cl, Ca, Fe, Rb) (Reid et al., 2005). Approximately 10% of the fine-mode mass of fresh smoke is composed of trace inorganic elements, most notably potassium, chlorine, and calcium, which are likely present in the particle core. Potassium and chloride each account for 2–5% of fine particle mass. It has often been suggested that they are present in the form of potassium chloride in the core of smoke particles with BC. Sulfur in the form of sulfate is also present at ~1% of fine particle mass (Reid et al., 2005).

BC is estimated to be the second or third largest warming agent after carbon dioxide in terms of direct radiative forcing, a metric used to compare the strength of greenhouse pollutants (Ramanathan and Carmichael, 2008; Van Vliet et al., 2013). Beelen et al. (2008) found evidence for an association of exposure to black smoke with lung cancer incidence in people who had never smoked. The BC exposure induces oxidative-inflammatory reactions and DNA damage in rats (Danielsen et al., 2010) and increase of biomarkers for inflammation in mouse pulmonary alveolar macrophage (Shang et al., 2013).

Gaseous sulfur dioxide (SO_2) is the precursor of sulfate in particles (Hou et al., 2013). Previous studies have observed association of increased lung cancer risk with occupational and environmental exposure to SO_2 (Lee et al., 2002; Tseng et al., 2012). DNA methylation has emerged as a promising biomarker for environmental-related diseases, including lung cancer. Hou et al. (2013) investigated the effects of elemental components on two groups of highly exposed individuals in Beijing, China. They found that altered DNA methylation in tandem repeat element was positively associated with concentrations of Si and Ca in truck drivers and S in office workers. Williams et al. (2012) found daily potassium air concentrations to be associated with decreased diastolic blood pressure in Detroit, MI.

Lara et al. (2005) analyzed the properties of aerosols from sugar-cane burning and found that emissions of elements such as K, S and Cl and $PM_{2.5}$ were 2–10 times higher in the biomass burning source profile. K, S and Cl were also identified in our samples and were the most abundant elements found. The high levels of Si may be associated with resuspended soil (Lara et al., 2005).

Among the five most abundant PAHs identified, three are considered carcinogenic/mutagenic (BkF, BbF and InP) and two are considered mutagenic (BghiP, Phe) (IARC, 2010). Carcinogenic PAHs (BbF, BkF, BaP, InP, and BaA) account for 59.6% of the PAH mass found in the Amarelão community. This result is higher than that found by Vasconcellos et al. (2011) in São Paulo.

Retene has been proposed as a tracer for conifer combustion sources (Ramdahl, 1983) and abundant amounts of phenanthrene, fluoranthene and pyrene have been found in biomass burning smoke (Simoneit, 2002; Vasconcellos et al., 2010). These PAHs were also identified in our samples. Phenanthrene was the fourth most abundant compound found, but the other compounds were not plentiful. This can be explained by the fact that cashew nut roasting represents a particular type of biomass burning. The shell of the cashew nut itself acts as the fuel and the workers do not use wood. The amount and composition of the numerous organic compounds released from biomass burning depend on various factors, such as fuel composition, burning rate, topography, terrain morphology and weather conditions (Khalil and Ramussen, 2003; Reche et al., 2012).

Benzo[a]pyrene-equivalent carcinogenic power (BaPE) is an index that has been introduced for better parametrizing aerosol carcinogenicity related to the whole PAH fraction instead of the sole benzo[a]pyrene, since the latter is easily decomposed in reactive air (Yassaa et al., 2001). It indicates the health risk for humans with respect to ambient PAH exposition and is calculated by multiplying the concentrations of each carcinogenic congener (Vasconcellos et al., 2010). The average BaPE value at the Amarelão community (1.94) was similar to that found by Vasconcellos et al. (2010) for sugar cane burning (2.1) and São Paulo City (1.9).

In this respect, the presence of high amounts of mutagenic and carcinogenic PAHs found in the PM collected in the Amarelão community, mainly, BkF, InP, BghiP, Phe and BbF, may have influenced genotoxicity, since these elements can potentially damage DNA.

The genotoxicity assay with *T. pallida* can be used as a screening tool to assess human risk under unfavorable environmental conditions (Sisenando et al., 2011; Carreras et al., 2013). The influence of weather conditions such as relative humidity, temperature and rainfall on the Trad-MCN assay as a result of exposure to genotoxic agents has been investigated by a number of studies (Klumpp et al., 2004; Savóia et al., 2009; Sisenando et al., 2011; Pereira et al., 2013). Corroborating Savóia et al. (2009) and Sisenando et al. (2011), we also observed an inverse relationship between the rate of MN and rainfall (Table 3).

The inverse correlation observed between the rate of MN and rainfall is likely explained by the fact that in the rainy season the aerosols suspended in the atmosphere are precipitated, reducing their concentration in the air. Thus, rainfall plays a positive role as a self-purifying agent of air pollutants. Studies in Brazil conducted by Maenhaut et al. (2002) and Lara et al. (2005) with aerosol emissions from burning biomass in tropical forests and sugar cane burning revealed a variation in the concentration of a number of air pollutants between the rainy and dry seasons. The mean concentration of $PM_{2.5}$, PM_{10}, BC and some chemical elements was lower in the rainy season than in dry season.

The rate of MN resulting from biomass burning by cashew nuts is higher than that obtained by several studies that used *T. pallida* in the Trad-MCN in order to evaluate the genotoxicity of various sources of air pollution (Prajapati and Tripathi, 2008; Mariani et al., 2009; Meireles et al., 2009; Savóia et al., 2009; Alves et al., 2011; Pereira et al., 2013).

Although there are no studies that use NPBs and NFs as biomarkers of DNA damage in *Tradescantia* tetrads, they have been used in binucleated human cells, particularly lymphocytes, and established cell lines. Research with human WIL2-NS cells (Umegaki and Fenech, 2000) and human lymphocytes (Fenech and Crott, 2002) observed an inter-relationship between the MN frequency and other biomarkers of DNA damage, such as nuclear budding and NPBs. The results for the Amarelão community also showed a positive correlation between MN frequency and other biomarkers used to assess genotoxicity in the Trad-MCN assay (Table 3).

A study carried out by Mariani et al. (2009) in São José dos Campos, Brazil showed that MN frequency in *T. pallida* exposed to urban air pollution was significantly associated to the mortality rate (deaths/10000 inhabitants) adjusted for cardiovascular diseases ($r=0.841$; $p=0.036$) and cancer ($r=0.890$; $p=0.018$) in the general population. Sisenando et al. (2011) evaluated biomass burning pollutants, showing a significant positive correlation between the standardized rate (hospital morbidity/1000 inhabitants) in children and %MN ($r=0.721$; $p=0.019$).

Further studies should also be performed to detect other types of air pollutants, in order to better characterize the trace elements of cashew nut burning (i.e. levoglucosan, mannosan and galactosan) and other potentially carcinogenic compounds (i.e. nitro-PAHs and oxy-PAHs), analyze their effects on the biota and the workers involved, in addition to better characterizing and understanding the genetic-molecular processes involved in the genotoxicity observed in cashew nut roasting.

5. Conclusions

Taken together, the results indicated genotoxicity for the Amarelão community, and the high rates of $PM_{2.5}$ are considered a potential contributor to this effect, mainly the high presence of mutagenic (Phe, BeP, BghiP) and carcinogenic (BaA, BbF, BkF, BaP, InP, DBA) PAHs. The biomass burning tracers K, S, Cl were the major inorganic elements found. The Trad-MCN using the *Tradescantia pallida* (*in situ*) was sensitive and efficient in detecting genotoxic damage by micronucleus analysis. Moreover, other nuclear alterations, such as nucleoplasmic bridges and nuclear fragments showed a higher correlation with micronuclei frequency. Environmental variables, such as rainfall, influenced genotoxicity.

Because of the importance of air quality and the social and economic significance of the cashew nut for thousands of families around the world, urgent action is required from public officials to promote the organization of associations and provide mini-factories with exhaust systems that eliminate air pollutants. Since worker health is essential to sustain this activity, it is inconceivable to allow it to continue in its current form.

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