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Determination of phytotoxicity and cytogenotoxicity due to exposure to particles originating from sugarcane burning using test systems *Lactuca sativa* L. and *Allium cepa* L.

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ABSTRACT

Sugarcane straw burning generates particulate matter with complex composition resulting in atmosphere pollution. Sugarcane straw sugarcane burning particles (PSSB) contain several chemical compounds that were previously identified to be associated with carcinogenic and mutagenic processes. The aim of the present study was to extract PSSB under lab conditions and subsequently determine phyto- and cytogenotoxicity of these particles using *Lactuca sativa* L. and *Allium cepa* L. bioassays. Seeds of lettuce var. Cinderela and onion cv. Vale-Ouro IPA-11 were germinated in Petri dishes containing different concentrations of PSSB at 25, 50, 100, 200 or 300 mg/ml as well as control for 72 hr. Seed germination of lettuce was inhibited by PSSB, in a concentration-dependent manner, accompanied by decreased root growth, suggesting phytotoxic effects. Further, reduction of mitotic index and high number of chromosomal alterations in onion of meristematics cells indicated a cytogenotoxic action attributed to PSSB. Although the chemical composition of PSSB in question has not been determined, the phyto- and cytogenotoxic effects may be linked to the possible presence of polycyclic aromatic hydrocarbons (PAHs), which are were identified as the main constituents of particulate matter resulting from burning of sugarcane straw, in addition to exerting adverse biological effects that might result in mutations and cancer. Data demonstrated that the use of plants bioassays might be an important tool for biomonitoring air quality.

KEYWORDS

Atmospheric pollution; particulate matter; toxicity; plant bioassays; chromosomal alterations

Introduction

The effects of air pollution continue to be a matter of global concern, predominantly due to the adverse effects of contaminants on human and environmental health (Bontinck, Maes, and Joos 2020; de Oliveira et al. 2014; Shahrbaf et al. 2021; Shkirkova et al. 2020). In terms of world population, approximately three billion individuals are exposed to air pollution attributed to emissions from biomass burning, resulting from different human actions such as deforestation, burning of crops for pre-harvest, as well as processing by agro-industries and mini-factories (de Oliveira Galvão et al. 2020; Phairuang et al. 2019; Santiago-De La Rosa et al. 2018; Weichenthal et al. 2017; WHO 2017).

In Brazil, sugarcane is an example of agribusiness in expansion, since this crop is considered an important alternative for production and consumption of biofuel related to production of ethanol and its by-products (CONAB [Companhia Nacional de Abastecimento (National Supply Company)] 2021; Sisenando et al. 2011). In the 2020/2021 cycle, Brazil harvested an area of 8,616.1 thousand hectares destined for the sugar and alcohol activity, with a productivity of 75,965 Kg/ha, resulting in a volume of sugarcane production of 654,527.8 thousand tons, a value higher than 1.8% compared to the 2019/2020 harvest. In a global scenario, these numbers rank Brazil in first place in the ranking of sugarcane producing countries, with the largest production concentrated in the Center-South region of the country (CONAB [Companhia Nacional de Abastecimento (National Supply Company)] 2021; Nachiluk 2021).

In the Submédio São Francisco Valley, the cities of Petrolina (PE) and Juazeiro (BA) lead an Integrated Economic Development Network

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(IEDN), termed Petrolina-Juazeiro Pole, together with the municipalities of Lagoa Grande, Santa Maria da Boa Vista and Orocó (PE), Casa Nova, Curaçá and Sobradinho (BA), configuring one of the main centers of irrigated fruit growing in Brazil (Oliveira 2015). In this Brazilian semiarid region, the high solar incidence, favorable humidity and irrigation system contributed to profitable production of sugarcane (Soares et al. 2003) and played a significant role in job creation and local economy (Ribeiro and Azevedo 2016). However, the adoption of non-mechanized harvesting generated local and neighboring cities disturbances attributed to emissions of particulate matter accumulating in urban and rural areas, mainly during the period from May – November (dry season) (Machin and Nascimento 2018; Škarek et al. 2007).

Although modernization has occurred in production of sugarcane in the Center-South region of the country, manual harvesting is still widely practiced in Brazilian production, especially in the North/Northeast region, where it reached 76.8% in the 2020/2021 harvest. In addition to being low-cost, this non-mechanized cutting system facilitates harvesting and increases the yield of manual cutting, which is carried out by pre-burning the sugarcane straw (CONAB [Companhia Nacional de Abastecimento (National Supply Company)] 2021).

Burning sugarcane straw releases various pollutants into the atmosphere including: fine ($PM_{2.5}$) and coarse (PM_{10}) particulate matter, gases such as monoxide (CO) and carbon dioxide (CO_2), acid esters fatty acids, aliphatic hydrocarbons, metals and PAHs (Arbex et al. 2004; de Andrade et al. 2010; Inácio and Brandão 2016; Zamperlini, Silva, and Vilegas 1997). These compounds are emitted and increase pollutant concentrations in the atmosphere (CONAB [Companhia Nacional de Abastecimento (National Supply Company)] 2021; Magalhães, Bruns, and Vasconcellos 2007; Moraes 2007; Sisenando et al. 2011; Sisenando et al. 2012; Martin et al. 2020), resulting in economic, social and environmental damage (de Andrade et al. 2010). At high concentrations or after prolonged exposure, these pollutants induce genotoxic and/or mutagenic effects not only to humans (de Oliveira Alves et al. 2017; Sisenando et al. 2012), but also to other animals (Meire, Azeredo, and Torres 2007), vegetables (Silva et al. 2012; Sisenando et al. 2011) and bacteria (Batistuzzo et al. 2016), compromising ecosystem health.

Among the pollutants, PAHs constitute a large proportion of the material resulting from biomass burning, including sugarcane straw (Silva et al. 2012), rice straw (Park et al. 2018), and cashew nut (de Oliveira Galvão et al. 2020). These compounds constitute a major contributor to hazardous biological effects attributed to particulate matter (Idowu et al. 2019; Ke et al. 2018). Epidemiological and toxicological studies suggest a strong link between the exposure of particles from biomass burning and adverse effects on human health associated with occurrence of oxidative stress and formation of DNA adducts, whose absence of repair may result in mutations and cancer (Abbas et al. 2013; Bornholdt et al. 2002; de Oliveira Alves et al. 2017; Iarmarcovai et al. 2008; Pardo et al. 2020; Sarigiannis et al. 2015; Zheng et al. 2018a).

Among the tests used to evaluate the genotoxic and/or mutagenic potential of atmospheric pollutants, environmental monitoring through use of various plant bioassays including models such as *Tradescantia pallida* (Rose) D.R.Hunt (purple queen), *Allium cepa* L. (onion) and *Lactuca sativa* L. (lettuce) (Campagna-Fernandes, Marin, and Penha 2016; Pereira, de Campos Júnior, and Morelli 2013; Silveira et al. 2017) were found to be effective. To assess the genotoxic potential of air pollutants, the micronucleus (MN) test in *T. pallida* (Trad-MCN) is considered a valuable tool. Sisenando et al. (2011) reported in Tangará da Serra (AM, Brazil), a high frequency of MN using *T. pallida* associated with the appearance of fine atmospheric particulate matter and increased hospital morbidity attributed to respiratory diseases in children during the dry season. Silva et al. (2012) found that residual particles from burning of sugarcane were identified as responsible for the rise in frequency of MN in *T. pallida* pollen grain mother cells affirming the high sensitivity of this bioassay to genotoxicants.

It is noteworthy that the test systems employing *L. sativa* and *A. cepa*, both accepted by environmental agencies (Grant 1999; OECD 2006; USEPA 1996), were utilized to assess the genotoxicity of

different environmental pollutants (Silveira et al. 2017). The lettuce bioassay analyzes the phytotoxic potential of the tested sample, by evaluating germination and root growth (Mtisi and Gwenzi 2019; Silva et al. 2017; Valerio, Garcia, and Peinado 2007), while the onion assay was employed to identify cytotoxicity, genotoxicity and mutagenic activity of contaminants (Freire et al. 2020; Leme and Marin-Morales 2009; Sousa et al. 2021).

Bearing this in mind the aim of the present study was to examine the possible phytotoxic and cytogenotoxic potentials of particles generated from sugarcane straw burning (PSSB) under lab conditions using *L. sativa* and *A. cepa* as model bioassays.

Materials and methods

Sugarcane collection and processing (Saccharum officinarum L.)

Sugarcane tips were collected manually with the aid of machetes and sickles, in an experimental area located at Campus Ciências Agrárias – UNIVASF, Petrolina/PE (09°19′12,0" S 40°33′44,3" W), in December/2019. Exsiccates were prepared and deposited at the Vale do São Francisco Herbarium (HVASF) (UNIVASF, Petrolina/PE), with the tombo number 24230. This project was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) under the number ADF11B8.

The sugarcane tips were collected and crushed in a forage crusher located in the production sector of the Campus Ciências Agrárias – UNIVASF, resulting in smaller sugarcane fragments, which were stored in nylon bags. This material was transported to the Cytogenetics Laboratory, located at the Molecular Ecology Center (NECMOL) of the Caatinga Fauna Management Center (CEMAFAUNA) – UNIVASF, where it was subjected to drying in an oven at 55°C for 72 hr.

Obtaining of particles from sugarcane biomass burning

The dry material was grounded in a forage, filtered and resulting residues incinerated in a muffle furnace, following the protocol of Document 236 – Procedures for Lignocellulosic Analysis – EMBRAPA (Morais, Rosa, and Marconcini 2010), with some modifications described below. The residue of the dry material was submitted to a muffle furnace at a temperature of 250°C for 30 min, with a heating ramp of 8°C/min, obtaining particles from surgacane straw burning (PSSB). The material obtained was stored in plastic pots sealed with parafilm at room temperature, for later use in toxicity and cytogenotoxicity testing.

Lactuca sativa and *Allium cepa bioassays*

Phytotoxicity assay

In order to verify a possible phytotoxic action of PSSB lettuce seeds var. Cinderella were exposed to different concentrations of particles (25, 50, 100, 200 03 300 mg/ml), as well as negative (ultrapure water) and positive controls [herbicid trifluralin (0.84 ppm of active principle), aneugenic action compound] and [methylmethane sulfonate; MMS ($4x10^{-4}$ M), compound of clastogenic action], totaling 8 treatments.

For each treatment, 120 seeds were placed in three Petri dishes (100 x 15 mm) (40 seeds/dish; three repetitions) lined with voile tissue (treatment with PSSB) or filter paper (negative and positive controls), containing 10 ml respective concentrations of PSSB, negative or positive controls Petri dishes kept at 25°C for 72 hr. Then, the number of germinated seeds was recorded and average length of 20 newly, randomly selected germinated roots measured.

From these data, the absolute germination (AG) and germination index (GI) were calculated, the latter used for classification of phytotoxicity (Paradelo et al. 2008). To obtain the AG, equation 1 was applied:

$$\%AG = \frac{Number\ of\ germinated\ seeds}{Number\ \ of total\ seeds} x100 \quad (1)$$

The GI was calculated based upon the quantification of relative seed germination (RSG) and relative rooth growth (RRG), according to Tiquia, Tam, and Hodgkiss (1996) and with modifications proposed by Vasconcelos, Oliveira, and França (2013). To calculate the % RSG, equation 2 was used:

Table 1. Parameters of the phytotoxicity and cytogenotoxicity of particles from surgacane straw burning (PSSB) using the bioassays with *Lactuca sativa* L. and *Allium cepa* L., respectively.

	Lactuca sativa L. bioassay				*Allium cepa* L. bioassay	
Treatments	AG (%)	ARL (cm)	GI (%)	Phytotoxicity*	MI (%)	CAI (%)
NC	96.67	1.84 ± 0.44	100	Absent	21.41 ± 8.38	0.21 ± 0.18
MMS	58.33	**0.50 ± 0.08**[a]	16.45	High	**11.66 ± 4.14**[a]	**0.85 ± 0.43**[a]
TRI	24.17	**0.45 ± 0.12**[a]	6.07	High	**15.59 ± 4.00**[a]	**1.72 ± 0.58**[a]
25 mg/mL	79.17	**0.47 ± 0.18**[a]	20.88	High	**6.18 ± 2.06**[a]	**0.46 ± 0.32**[a]
50 mg/mL	71.67	**0.40 ± 0.12**[a]	16.08	High	**6.29 ± 2.73**[a]	**0.41 ± 0.16**[a]
100 mg/mL	74.17	**0.40 ± 0.08**[a]	16.64	High	**9.84 ± 6.14**[a]	**0.82 ± 0.69**[a]
200 mg/mL	65.83	**0.45 ± 0.17**[a]	16.70	High	**6.50 ± 3.70**[a]	**0.55 ± 0.35**[a]
300 mg/mL	30.83	**0.29 ± 0.08**[a]	5.06	High	**6.93 ± 2.64**[a]	**0.68 ± 0.37**[a]

Subtitle: Data presented in percentage or in mean and standard deviation. NC – Negative Control; MMS – Methyl MethaneSulfonate; TRI – Trifluralin herbicide; AG – Absolute Germination; ARL – Average Root Length; GI – Germination Index; * considering the classification proposed by Paradelo et al. (2008); MI – Mitotic Index; CAI – Chromosomal Alterations Index; [a]Significantly different from NC ($p < 0.05$).

$$\%RSG = \frac{\bar{N}_{GS,T}}{\bar{N}_{GS,NC}} x100 \quad (2)$$

Where, $\bar{N}_{GS,T}$ means the average number of germinated seeds in the respective treatments, while $\bar{N}_{GS,NC}$ refers to the average number of seeds germinated in the negative control. To calculate the % RRG, equation 3 was applied:

$$\%RRG = \frac{\bar{C}_{R,T}}{\bar{C}_{R,NC}} x100 \quad (3)$$

Where, $\bar{C}_{R,T}$ is the average length of the treatment roots and $\bar{C}_{R,T}$ is the average length of the newly germinated roots of the negative control.

After obtaining the %RSG and %RRG values, the %GI was calculated using equation 4:

$$\%GI = \frac{(\%RSG)X(\%RRG)}{100} \quad (4)$$

For the classification of phytotoxicity, the GI was used following the classification by Paradelo et al. (2008), as presented in Table 1.

Cytogenotoxicity assay

The cytogenotoxic potential of PSSB was tested with the test organism *A. cepa*. A total of 150 onion seeds IPA-11 were germinated in Petri dishes (100 x 15 mm) with different concentrations of PSSB (25, 50, 100, 200 or 300 mg/ml) or with positive and negative controls, described in the assay with *L. sativa*. After 72 hr of exposure, 20 newly, randomly collected germinated roots were examined, fixed in ethanol:acetic acid solution (3:1) for 6 to 8 hr at room temperature, and subsequently stored at – 20°C. The slides were prepared according to the procedure of Fiskesjö (1985), with adaptations according to Fernandes, Mazzeo, and Marin-Morales (2007). The roots were removed from the fixative and the excess was removed with the aid of filter paper. Then, these roots were washed in distilled water, hydrolyzed in 1 N HCl at 60°C for 10 min, washed and stained with Schiff's Reagent (1.090033, Sigma-Aldrich) for 2 hr in the dark. After this duration, roots were washed again in distilled water and placed on a slide.

With the use of a scalpel, the meristematic tissue region was identified and placed on the slide for cell counting. For cell contrast, a drop of 2% acetic carmine was placed on the material, which was covered with a cover slip and slightly crushed, in order to spread the cells without compromising cell integrity. The slides were then buckled and mounted with Entellan® (107960, Merck Milipore). The analysis of the slides was performed under an optical light microscope with a total magnification of up to 400x magnification, in order to facilitate the identification and counting of cellular anomalies.

In this bioassay, the mitotic index (MI) and chromosomal alterations index (CAI) were obtained from analysis of 500 meristematic cells/slide performed from reading of 10 slides/treatment, totaling 5000 cells/treatment. The images of CAI were captured using a Leica DM2500 epifluorescence microscope coupled to a Leica DFC345 FX camera and Leica LASX software optimized for clearer brightness and contrast with Adobe Photoshop CS4 (Adobe Systems Incorporated).

Statistical analysis

For the *L. sativa* and *A. cepa* bioassays, 8 treatments were evaluated as follows: 5 different PSSB concentrations, one negative and two positive controls. Frequency values were transformed using the formula (arcosine √ %). Data normality was verified by the Shapiro-Wilk test, while homogeneity was tested by ANOVA followed by Levene's test. After this analysis, the Student's t-test (parametric) and Mann-Whitney (non-parametric) were applied to assess the AG and average root length (ARL) obtained in the bioassay with *L. sativa*, as well as to verify the MI and CAI obtained in the bioassay with *A. cepa*, using the Statistic 8.0 software. The criterion for significance was set at $p < .05$.

Results

Phytotoxicity analysis on the Lactuca sativa L.

The effects of PSSB on seed germination and root growth of lettuce, as well as the phytotoxicity classification, are presented in Table 1. Considering the GA, there was a reduction in the germination rate of lettuce seeds compared to negative control (96.67%) (Table 1), which was inversely proportional to PSSB concentrations tested, ranging from 79.17% (25 mg/ml PSSB) – 30.83% (300 mg/ml PSSB). Similarly, the ARL values were significantly decreased in size of germinated roots for all treatments, ranging from 0.47 cm (25 mg/ml PSSB) – 0.29 cm (300 mg/ml PSSB), compared to negative control (1.84 cm) (Table 1). Thus, both parameters indicated the presence of toxicity of attributed to PSSB exposure in *L. sativa* seeds, which occurred in a concentration-dependent manner.

This PSSB observed toxicity was corroborated by GI values obtained, which were calculated as a function of RSG and RRG. The GI ranged from 20.88 (25 mg/ml PSSB) to 5.06% (300 mg/ml PSSB) values classified as highly phytotoxic (Paradelo et al. 2008) (Table 1), emphasizing that these particles resulting from burning of sugarcane adversely affected seed germination and growth of newly germinated roots in lettuce.

Cytogenotoxicity evaluation on the Allium cepa L.

The influence of PSSB on MI and CAI are summarized in Table 1. A cytotoxic action of PSSB was evidenced in meristematic cells of *A. cepa*, attributed to reduction in MI for all PSSB treatments compared to negative control (21.41%) with the least (6.18%) and higher MI (9.84%) noted for treatments with 25 and 100 mg/ml PSSB, respectively (Table 1). Further, a significant increase in CAI was detected for PSSB tested at 25 mg/ml (0.46%), 50 mg/ml (0.41%), 100 mg/ml (0.82%), 200 mg/ ml (0.55%) and 300 mg/ml (0.68%), compared to negative control (0.21%) (Table 1) with MN, nuclear buds, chromosomal bridges and losses predominantly noted (Figure 1; Table 2).

Discussion

Biomass burning releases several pollutants into the atmosphere and these compounds have been associated with adverse effects on human health and the ecosystem (de Oliveira Alves et al. 2011; Martin et al. 2020). Among the biological assays proposed to investigate the toxicity of atmospheric environmental pollutants, plants bioassays are considered important for their simplicity, low cost and high sensitivity (Grant 1994, 1999). However, the responses assessed for toxicity and ecotoxicity tests depend upon (1) bioassay indicator(s) used, (2) physiological and/or genetic parameters evaluated in each assay, such as AG, root length and alterations at cell cycle and/or chromosomal level, and (3) compound to be investigated. This fact is related to the intrinsic natural differences of test organisms, whose sensitivity to chemical compounds may vary between different taxonomic groups, even within the same species (Wang and Freemark 1995), which support the extensive use of *L. sativa* as model in phytotoxic investigations (Charles et al. 2011; Tigre et al. 2012), while *A. cepa* is most commonly applied for cytogenotoxic tests (Freire et al. 2020; Grant 1999; Issa et al. 2020; Laughinghouse et al. 2012; Sousa et al. 2021; Yildiz et al. 2009).

In this study, data demonstrated that PSSB exerted phyto- and cytogenotoxic effects using *L. sativa* and *A. cepa* systems, respectively. The inhibitory effects of PSSB on germination and root

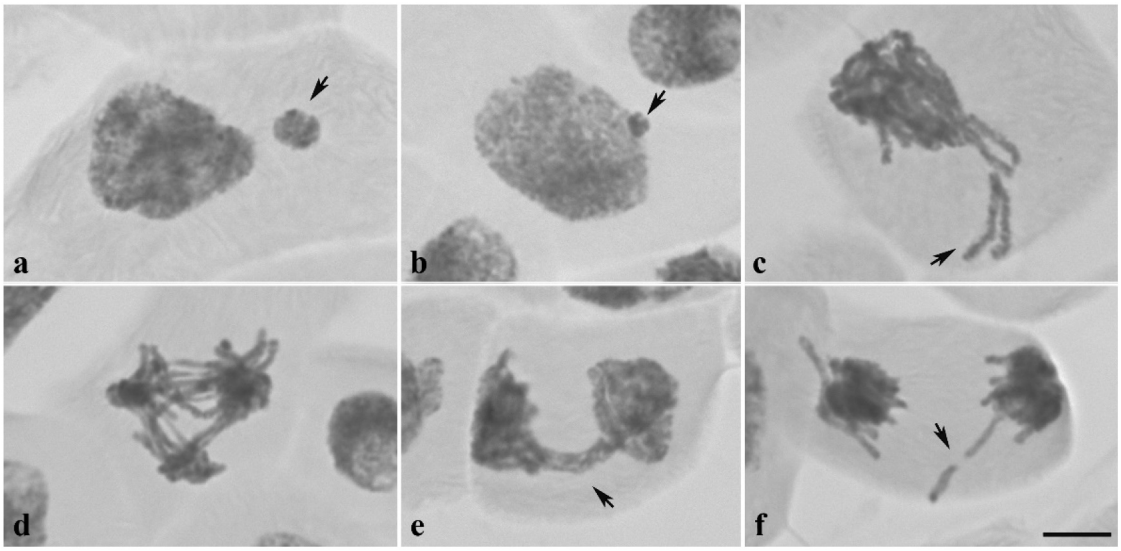

Figure 1. Chromosomal alterations observed in meristematic cells of *Allium cepa* L., used as parameters of genotoxicity of particles resulting from sugarcane burning. Subtitle: **a**. Micronucleus (arrow). **b**. Nuclear bud (arrow). **c**. Chromosome loss (arrow). **d**. Multipolar anaphase. **e**. Telophase bridge (arrow). **F**. Telophase with chromosome break. Scala (in f) = 7,5 µm.

Table 2. Frequency of chromosome alterations in meristematic cells of *Allium cepa* L. after exposure to the particles from surgacane straw burning (PSSB).

	Frequency of chromosome alteration (%)								
Treatments	MN	BN	PC	PT	CL	QC	AM	AC	CB
NC	0.16 ± 0.16	0.02 ± 0.05	0.02 ± 0.05	0 ± 0	0 ± 0	0.02 ± 0.05	0 ± 0	0 ± 0	0 ± 0
MMS	**0.50 ± 0.32**[a]	0.13 ± 0.18	0.07 ± 0.12	0.09 ± 0.15	0.02 ± 0.05	0 ± 0	0.02 ± 0.06	0.02 ± 0.05	0 ± 0
TRI	**0.77 ± 0.55**[a]	**0.36 ± 0.41**[a]	**0.12 ± 0.11**[a]	**0.34 ± 0.32**[a]	0 ± 0	0.02 ± 0.06	0.05 ± 0.10	0.06 ± 0.18	0 ± 0
25 mg/mL	0.26 ± 0.30	0.08 ± 0.11	0.05 ± 0.08	0.05 ± 0.08	0.02 ± 0.05	0 ± 0	0 ± 0	0 ± 0	0 ± 0
50 mg/mL	0.34 ± 0.20	0.07 ± 0.09	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
100 mg/mL	0.46 ± 0.40	**0.23 ± 0.26**[a]	0.02 ± 0.06	0.06 ± 0.09	0.04 ± 0.12	0.02 ± 0.06	0 ± 0	0 ± 0	0 ± 0
200 mg/mL	0.36 ± 0.25	0.06 ± 0.08	0.04 ± 0.07	0.04 ± 0.08	0.02 ± 0.06	0 ± 0	0.02 ± 0.06	0.02 ± 0.05	0 ± 0
300 mg/mL	0.32 ± 0.23	0.23 ± 0.30	0 ± 0	0.05 ± 0.08	0.02 ± 0.05	0.02 ± 0.06	0.02 ± 0.06	0.01 ± 0.04	0.02 ± 0.05

Subtitle: Data presented in percentage or in mean and standard deviation. NC – Negative Control; MMS – Methyl MethaneSulfonate; TRI – Trifluralin herbicide; MN- Micronuclei; BN- Nuclear bud; PC- Chromosome loss; PT- Chromosome bridge; CL – Laggard chromosome; QC- Chromosome break; AM- Multipolar anaphase; AC- Chromosome adherence; CB- Binucleated cell. [a]Significantly different from NC ($p < 0.05$).

growth of lettuce seeds indicated phytotoxicity, whereas the mitodepressive effects on the cell cycle and induction of chromosomal alterations on onion cells indicated cytogenotoxicity. The germination data in PSSB showed an adverse interference of these particles on growth of *L. sativa* roots. Mtisi and Gwenzi (2019) observed a similar result when examining bioavailability of metals and phytotoxicity of coal ash in *L. sativa*, demonstrating that higher concentrations of coal ash applied to the soil (50 and 75%) reduced root length. These findings contradict those suggesting that exposure to coal ash is beneficial for the initial development of plants (da Costa et al. 2018; de Sousa et al. 2017). Although the consequences of contaminant exposure to *L. sativa* remains controversial, evidence indicates that treatment with PSSB inhibited AG and root length growth affirming that this plant bioassay was sensitive to detect phytotoxicity. In general terms, the adverse interference of toxic substances on root growth termed sublethal effects, occurs at low concentrations and enables the realistic establishment of toxic chemical concentrations (Campagna-Fernandes, Marin, and Penha 2016).

In lettuce, sensitivity may be related to rapid seed germination, high proliferation rate and linear root growth, favoring acquistion and analysis of data in a rapid manner (Aragão et al. 2017; Grant 1994). This bioassay is employed for detection/evaluation of allelochemicals with promising use as bioherbicides. The main parameter assessed is root elongation (Campos et al. 2008), a reliable, sensitive

indicator of environmental exposure and toxicity (Ratsch and Johndro 1986). It is worth noting that the intrinsic characteristics of this species resulted in application of this bioassay as a bioindicator to determine phytotoxicity of samples of water and sediment (Priac, Badot, and Crini 2017), metals (Bagur-González et al. 2011), extracts and vegetable oils (Andrade-Vieira et al. 2014; Sousa and Viccini 2011), environmental stresses such as high salt concentrations (Campagna-Fernandes, Marin, and Penha 2016), medicines (Rede et al. 2019), as well as atmospheric PM (França et al. 2017; Zheng et al. 2018a). In agreement with these observations the *L sativa* bioassay was sensitive to identify PSSB-mediated phytotoxicity as evidenced in this investigation.

Data demonstrated that PSSB disrupted the cell cycle and elevated number of chromosomal aberrations using *A. cepa* indicative of a cytogenotoxic action in agreement with previous findings (Park et al. 2018; Silva et al. 2012). Silva et al. (2012) reported a genotoxic action as evidenced by increased frequency of MN in *T. pallida* pollen grain mother cells, exposed to particles from sugarcane burning residues, which resembled toxicity induced by particles derived from fuels fossils. Park et al. (2018) noted a cytotoxic activity of PM resulting from burning of rice straw and pine stalk biomass as evidenced by (1) decrease in viability of human airway epithelial cell lines (A549, H292, BEAS-2B and SAEC), and (2) by diminished uptake of neutral red (NR) and water-soluble tetrazolium salt (WST-A), supporting an association between exposure to fine particles and adverse cellular effects.

According to Leme and Marin-Morales (2009), the decrease in MI indicates interference in transition of the phases of the mitotic cycle by chemical agents, preventing cell proliferation and reducing root growth, since the growth of an organ is related to sucessive cycles of cell division (Harashima and Schnittger 2010; Sousa and Viccini 2011; Taiz and Zeiger 2006). Such a relationship between MI and root growth was previously reported in cytotoxicity studies involving plant extracts (Aragão et al. 2017; Chiavegatto et al. 2017; Sousa and Viccini 2011). Thus, it is possible that the reduction in root growth observed in lettuce and MI in onion cells indicates the presence of mitodepressive substances in PSSB.

A directly proportional relationship was found between fall in MI and CAI in meristematic cells of *A. cepa*, which affirmed the genotoxic action of PSSB. The elevated frequency of chromosomal alterations may have been induced by different chemical compounds present in the composition of PSSB, which led to DNA breaks, inhibition of DNA synthesis and altered DNA replication (Claxton and Woodall 2007). However, identification of toxic compounds within PM is a challenge due to (1) complexity of the composition, (2) number of compounds present and (3) possible synergistic and antagonistic interactions (Jarvis et al. 2014).

Plants can incorporate PAHs through foliar uptake by dry deposition of particles in the aerial parts of the plant or through moisture precipitation (Wang et al. 2011). Thus, most of these compounds are present in leafy vegetables, rather than roots, which results in airborne rather than soil exposure (Tuteja, Rout, and Bishnoi 2011). However, Araújo et al. (2021) analyzed the influence of benzo(a) pyrene (PAH) on *L. sativa*, noting the presence of this contaminant in leaves but, more frequently in the roots, relating this to inhibition of growth of lettuce roots. These findings provide the assumption that the decline in AG and ARL in lettuce observed in our study, may be associated with the presence of these hydrocarbons in the particulate material tested.

The mechanism of action of PAHs has been extensively investigated and it is known that these compounds activate a series of enzymatically catalyzed reactions forming reactive metabolites, which covalently bind to purine DNA bases leading to formation of DNA adducts and, consequently to strand breaks (Jarvis et al. 2014; Santibáñez-Andrade et al. 2017), as well as interfering with DNA methylation, histone modification and oxidative stress inducing mutations and development of cancer (de Oliveira Galvão et al. 2020; Niranjan and Thakur 2017). Previous investigators demonstrated that exposure to PAHs present in soot results in respiratory diseases, cardiovascular disorders and cancer (Niranjan and Thakur 2017), which affect rural workers directly involved in cutting and burning from sugarcane (Ceccato et al. 2014; Matos, Fratari, and Carvalho 2018) as well as individuals exposed to pollutants present in the atmosphere

(Lemos et al. 2020; Sisenando et al. 2012). Sisenando et al. (2012) reported a rise in frequency of MN in the oral cells of children, aged between 6–16 years, exposed to air pollutants in Tangará da Serra (Amazon Region/Brazil), a region characterized by deforestation and large production of sugarcane non-mechanized sugar. Further, de Oliveira Alves et al. (2017); (2020)) noted an elevation in levels of reactive oxygen species (ROS), inflammatory cytokines, DNA damage, cell cycle alterations and cell death in human lung epithelial cells (A546 cell line) exposed to PM smaller than 10 μm (PM_{10}) emitted during biomass burning and cashew nut roasting in the Amazon region. In both cases, molecular and cellular toxic effects were attributed to the action of PAHs including dibenzo [a,h] anthracene, nitrated and oxygenated PAHs as well as to retene PAH, which was not included in the risk assessment of PAHs by US Environmental Protection Agency (USEPA) (de Oliveira Alves et al. 2017). However, the chemical composition of the particulate material under study was not analyzed; and thus one cannot attribute adverse effects of PSSB in seed germination and root growth of *L. sativa* and in cell cycle and chromosome level changes in *A. cepa* cells to specific toxic elements present in the particulate material.

Conclusions

The use of both test organisms *L. sativa* and *A. cepa* affirms the fact that both species displayed high sensitivity to environmental pollutants (Silveira et al. 2017), where *L. sativa* exhibits greater sensitivity to phytotoxic damage and *A. cepa* for cytogenotoxic potential. The phyto – and cytogenotoxicity of PSSB found in this study indicates that the action of chemical compounds that are toxic to the environment alo exert adverse consequences on plant species. The results emphasize an interference of particulate material on germination of lettuce seeds and root growth, as well as on cell division processes leading to formation of chromosomal alterations in onion meristematic cells. The use of model organisms *L. sativa* and *A. cepa* might serve as important tools to monitor air quality in regions that do not have environmental monitoring stations such as Petrolina (PE) – Juazeiro (BA) Pole.

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Data availability statement

Data supporting the findings of this study are available from the corresponding author, C.V., upon reasonable request or acessing doi: 10.6084/m9.figshare.19388678

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