

Morphophysiological changes in *Psidium cattleianum* caused by air emissions from a mining industry in Brumado, Bahia, Brazil

Katielle Silva Brito Kateivas¹, Paulo Araquém Ramos Cairo², Pedro Henrique Santos Neves³, Roger Sebastian Silva Ribeiro², Carlos André Espolador Leitão², Leohana Martins Machado²

¹ Instituto Federal de Educação, Ciência e Tecnologia Baiano, Itapetinga, BA, Brasil. E-mail: katielle_brito@yahoo.com.br (ORCID: 0000-0003-0847-289X)

² Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista, BA, Brasil. E-mail: pcairo@uol.com.br (ORCID: 0000-0002-3619-7867); ribeiro.roger.silva@gmail.com (ORCID: 0000-0002-4873-0892); candreel@yahoo.com.br (ORCID: 0000-0002-1988-7436); leohanamartins@hotmail.com (ORCID: 0000-0003-0381-9102)

³ Universidade Federal de Viçosa, Programa de Pós-Graduação em Fisiologia Vegetal, Viçosa, MG, Brasil. E-mail: pedrohenrique.uesb@gmail.com (ORCID: 0000-0001-7739-128X)

ABSTRACT: *Psidium cattleianum* is a bioindicator species of air pollutants, notably NO_x and SO₂. The aim of this study was to evaluate the effects of air emissions from a mining industry in Brumado, Bahia, Brazil, on the morphophysiological characteristics of *P. cattleianum*. A field experiment was carried out with potted plants in two distinct areas: (1) permanent exposure to NO_x and SO₂ emissions; and (2) free from the influence of the plume formed by these air pollutants (control). Plant height, leaves area and quantity, leaves, stem and roots dry mass, and chlorophyll and soluble sugar contents were evaluated. In the plants exposed to the pollutant plume, height growth inhibition and leaf size and quantity reduction were observed, besides showing symptoms of chlorosis and necrosis. Dry matter production was also lower in all organs. The chlorophyll a content was lower, but chlorophyll b content did not change. The soluble sugars content was not affected by the pollutants. It was concluded that the atmospheric emissions from the mining industry negatively affect the physiological characteristics of *P. cattleianum*, inhibiting the plant growth.

Key words: araquá; bioindicators; environmental pollution; growth; phytotoxicity

Alterações morfofisiológicas em *Psidium cattleianum* causadas por emissões atmosféricas de uma indústria mineradora em Brumado, Bahia, Brasil

RESUMO: *Psidium cattleianum* é uma espécie bioindicadora de poluentes atmosféricos, notadamente NO_x e SO₂. Neste estudo se objetivou avaliar os efeitos de emissões atmosféricas provenientes de uma indústria mineradora em Brumado, Bahia, Brasil, sobre características morfofisiológicas de *P. cattleianum*. Conduziu-se um experimento de campo, com plantas em vasos, em duas áreas distintas: (1) exposição permanente às emissões de NO_x e SO₂; e (2) livre da influência da pluma formada por esses poluentes atmosféricos (controle). Avaliaram-se altura de plantas, área foliar, número de folhas, massa seca de folhas, caule e raízes e teores de clorofilas e açúcares solúveis. Nas plantas expostas à pluma de poluentes, observaram-se inibição no crescimento em altura e redução no tamanho e quantidade de folhas, além de sintomas de clorose e necrose. A produção de massa seca também foi menor, em todos os órgãos. O teor de clorofila a foi mais baixo, mas o de clorofila b não se alterou. O teor de açúcares solúveis não foi afetado pelos poluentes. Concluiu-se que as emissões atmosféricas provenientes da indústria mineradora afetam negativamente características morfofisiológicas de *P. cattleianum*, inibindo o crescimento das plantas.

Palavras-chave: araquá; bioindicadores; poluição atmosférica; crescimento; fitotoxicidade

Introduction

Since the industrial revolution, the growing process of industrialization has led to a sequence of environmental degradation throughout the planet, with a significant increase of pollutants released into the atmosphere. In Brazil, the inefficient control of emissions and gaps in the legislation, which cause serious environmental problems, worsen the problem.

In the municipality of Brumado, located in the southwestern of Bahia, since the 1940s, Magnesita Mineração SA extracts and process the deposits of magnesite in Serra das Éguas. For the processing of this ore, the operation of the furnaces and emissions of particulate matter - nitrogen (NO_x) and sulfur (SO_2) oxides - are the main source of generation of agents potentially causing atmospheric pollution.

Pollution is a type of contamination that results in adverse biological effects on the environment. Depending on the physical and chemical characteristics of the pollutants, the permanence, concentration and dispersion of gases in the atmosphere may be influenced by the annual seasonality of meteorological factors such as temperature, humidity and precipitation. The influence of seasonality on air quality has been investigated in relation to its effects on human health (Ikram et al., 2015) and on the physiological characteristics of plant species (Moraes et al., 2002, Prajapati & Tripathi, 2008).

The ever-growing problems caused by environmental pollution are the main reason for the growing interest in finding more appropriate environmental monitoring methods, in order to detect the level of atmospheric contamination and its effects on living organisms in general. Due to the inherent limitations of assessing physical and chemical parameters of the atmosphere, biological indicators have been increasingly used as an alternative for monitoring environmental impacts in different ecosystems (Rai, 2016). The main advantages of using plant bioindicators in environmental monitoring programs are the low cost and the possibility of their use in the cumulative evaluation of events of a given period of time, retrieving information on environmental history that can not be detected or measured by other methods.

Plants accumulate and incorporate into their systems the environmental pollutants to which they are constantly exposed. Depending on their level of sensitivity, they may exhibit visible changes, including those in biochemical processes or accumulation of certain metabolites (Agbaire & Esiefarienrhe, 2009). The interactions between the effects of air pollutants on plants and of environmental factors can be complex. Temperature, humidity, light radiation, precipitation and edaphic characteristics can considerably affect the damage caused by pollutants to plants, often through changes in stomatal opening. On the other hand, atmospheric pollutants can alter the response of plants to environmental stress, accentuating the injuries (Bell, 2017).

Psidium cattleianum is a fruiting shrub that occurs in

Brazil from Bahia to Rio Grande do Sul. Among the different species of *Psidium*, *P. cattleianum* is considered one of the best for the production of araçá, a fruit of considerable nutritional value, due to low sugar levels and high content of phenolic compounds, vitamins and minerals, which preserve 45 % of vitamin C content (Franzon et al., 2009). *P. cattleianum* also attracts the interest of the pharmaceutical industry for its antioxidant substances (Franzon et al., 2009), and is considered as an alternative for family farming. It is an excellent option for organic cultivation in view of the characteristics of its fruits and good acceptance for consumption (Barbieri, 2011).

P. cattleianum cultivation is still modest from the economic point of view, in the context of national fruit farming. On the other hand, this species has been increasingly used as bioindicator in environmental monitoring programs of several atmospheric pollutants (Moraes et al., 2002; Perry et al., 2010). It is a bioindicator with long-term responses that lead to changes in biomass allocation and visible symptoms such as chlorosis and necrosis, besides having high capacity to accumulate minerals such as iron and sulfur (Moraes et al., 2002).

The literature has reports of phytotoxicity caused by air pollutants in araçá trees and other bioindicator species. Changes in the levels of chlorophylls (including symptoms of chlorosis and necrosis), sugars, proteins and other organic components (Thawale et al., 2011; Rai, 2016) are some physiological effects that may affect biomass production and plant growth. Studies on *P. cattleianum* submitted to the effects of pollutants in Brazil have revealed significant alterations in the antioxidant metabolism and vegetative growth of this plant (Moraes et al., 2002; Alves et al., 2011).

The objective of the present study was to evaluate possible morphophysiological changes in *P. cattleianum* plants subjected to continuous exposure to the pollutant plume created by atmospheric emissions resulting from the processing of magnesite ore by a mining industry located in the municipality of Brumado, southwest of Bahia, Brazil.

Material and Methods

Characterization of the area and the experiment

The Magnesita Mineração SA company is located in the municipality of Brumado (14°14'20" S and 41°43'31" W), located in the southwest of Bahia, Brazil. The company is in the northeastern semi-arid region whose climate is considered dry, of BSh type, according to Köppen, presenting a negative water balance resulting from an annual rainfall that ranges from 450 to 550 mm, with average insolation of 2,500 hours year⁻¹, mean temperature of 24 °C, annual evapotranspiration of 1,200 mm year⁻¹, and average relative humidity around 50% (INMET, 2016). The rainfall regime is marked by scarcity, irregularity and concentration of rainfall in only four months. During the field evaluations, in the present experiment, no precipitation was recorded in the study areas.

The experiment had a completely randomized design, and was carried out in two areas in the surroundings of Magnesita Mineração SA. The treatments had 14 repetitions and were represented by the constant exposure of plants to two different conditions of atmospheric pollutant concentration. The area 1 (14°14'36.1" S and 41°44'40.3" W) was exposed to atmospheric emissions of NO_x and SO₂, from mining industrial activity. The area 2 (14°113'37.8" S and 41°43'08.6" W, located about 3.5 km from area 1, is evidently free from the influence of the plume formed by these pollutants due to the direction of the winds, which prevents the dissipation of atmospheric emissions from industrial activity (Figure 1). The technical report on a study of the dispersion of atmospheric pollutants (particulate material, SO₂ and NO_x) in the surroundings of the industrial enterprise was considered for the selection of these areas, using the mathematical modeling made by a work and environmental safety company (SEGMA, 2010).

Two-month-old *P. cattleianum* seedlings were obtained from a commercial nursery and, after transported to the city of Brumado, they remained for two weeks in a nursery at Magnesita Mineração SA for acclimatization. At the end of October 2015, the seedlings were transplanted in 50 L pots (1 plant^{pot⁻¹}) and distributed in the field, in a previously cleaned area and under full exposure to sunlight, with a spacing of 2 m x 2 m. The pots were filled with previously fertilized soil based on the chemical analysis and nutritional requirements of the species, mixed with bovine manure, in a 3: 1 ratio. The plants were irrigated daily with sufficient water supply to leave soil moisture close to saturation in order to avoid that eventual water stress could constitute another variable influencing the results, due to seasonal meteorological oscillations.

Characteristics evaluated

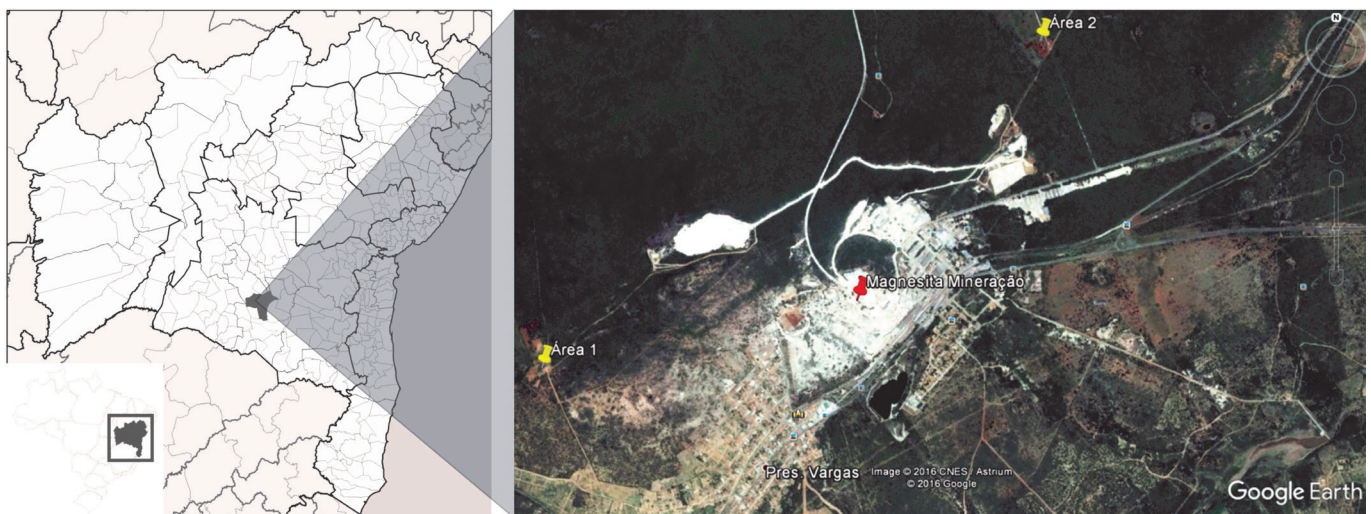
Characteristics of the seedlings that are measurable through non-destructive methods such as plant height and

chlorophyll content were evaluated from 120 days after the transplanting of the seedlings onwards. These evaluations were made once a month in February and March (summer) and from June to August (winter), considering the hypothesis that seasonal meteorological variations can influence the concentration and dispersion of pollutants in the atmosphere, altering its effects on the morphophysiological characteristics of the plants. Plant height was measured according to the length between the base and the apex of the stem, and chlorophyll content (SPAD index) was estimated in fully expanded and mature leaves of the medial portion of the treetop, using a chlorophyllometer (Minolta SPAD/502).

The pots were removed from the experimental areas in September 2016 (late winter) and transported to the Plant Physiology Laboratory of the State University of Southwest of Bahia, in Vitória da Conquista, where the plants were removed from the pots for evaluations of characteristics that require destructive methods.

Each plant had the number of leaves counted and the total leaf area measured by means of a Leaf Area Meter (LI-COR, LI-310). To calculate the leaf necrosis index, one leaf of each plant was collected, photographed, and printed on A4 paper (21.0 x 29.7 cm). The contours of the printed images of the leaves and the necrotic parts were cut out and their respective weights were measured. The areas of each leaf and of the necrotic parts were estimated by comparison with the respective weight of the A4 paper. The leaf necrosis index was expressed as percentage of necrotic areas in relation to the total leaf area.

Chlorophyll a and b were extracted from adult leaves, based on Arnon (1949). The procedure was carried out in a dark environment, illuminated only by green light, in order to avoid photo-oxidation of the pigments. After reading the chlorophyll extracts in a spectrophotometer, the values obtained were used in the formulas below, according to the model adjusted by Wellburn (1994), to obtain chlorophyll content, expressed in mg.g fresh mass⁻¹.



Source: Google Earth

Figure 1. Location of experimental areas. Magnesita Mineração S. A, Brumado, Bahia, Brazil.

$$\text{Chlorophyll a} = 12.25 A_{663} - 2.79 A_{647}$$

$$\text{Chlorophyll b} = 21.5 A_{647} - 5.1 A_{663}$$

Afterwards, the plants were fractionated in leaves, stems and roots, and later oven dried at 70 °C. Soluble sugars were extracted from leaves and roots, and their contents were quantified through the method proposed by Yemm & Willis (1954).

The means obtained in all measurements were compared using the t test ($p < 0.05$), in the statistical software SAEG, version 9.1, 2007.

Results and Discussion

The morphological characteristics evaluated were affected by exposure of the plants to the plume of pollutants, which inhibited their growth. From the second evaluation in March 2016 onwards, the plants exposed to the plume had lower growth in height than the control plants (Table 1). The negative effect of pollutants on plant height became increasingly evident, depending on the time of exposure to pollutants.

Some studies have demonstrated the harmful effects of prolonged exposure of plants to atmospheric pollutants (Furlan et al., 2007; Casimiro et al., 2016). For example, *Psidium guajava* plants presented increasing percentage of leaf injuries when exposed to O_3 , with symptoms reaching 100% of the plants after 60 days of exposure to this pollutant (Furlan et al., 2007). Furthermore, environmental factors such as air temperature and humidity may affect stomatal conductance, resulting in different intensities of responses to O_3 (Furlan et al., 2007; Alves et al., 2011).

The concentrations of pollutants such as SO_2 and NO_2 tend to increase in the atmosphere during the winter as a result of thermal inversion, which hinders the dispersion of gases (Moraes et al., 2002; Prabapati & Tripathi, 2008; Bulbovas et al., 2015). In the present study, the difference in height between the two treatments became more significant in the winter (July to September) (Table 1), suggesting that seasonal meteorological conditions may have affected the intensity of the effects of the pollutant on plant growth.

Inhibition of growth is an important indicator of harmful effects of air pollution on plants. This effect can be attributed to excessive concentrations of gases in the atmosphere, such

Table 1. Mean height of *Psidium cattleianum* plants grown in the control area and under exposure to pollutants released from industrial mining activity at different evaluation moments. Brumado, Bahia, Brazil.

Evaluation moments	Mean height of plants (cm)	
	Control	Plume
February	58.79 a	54.93 a
March	63.21 a	57.14 b
July	70.50 a	60.21 b
August	71.29 a	60.57 b
September	72.36 a	62.00 b

Means followed by the same letters, in the same line, indicate absence of significant differences, according to the t test ($p < 0.05$).

as SO_2 and NO_x (De Temmerman et al., 2004). Studies have shown that when these two gases act together, they can amplify their effects on plants, causing injuries on leaves and inhibition of physiological processes important for growth, such as photosynthesis and transpiration (Ashenden & Williams, 1980).

The leaf area and the number of leaves were negatively affected, and with great intensity, by exposure to the pollutant plume (Table 2). In the plants exposed to the pollutant plume, the values of these two morphological characteristics were 91.3% and 84.9% smaller, respectively, than those obtained in the control plants.

Similar results were found in studies on the influence of phytotoxic pollutants emitted by a fertilizer factory in Udaipur, India on young guava plants (*Psidium guajava* L.), a shrub species (*Carissa carandas* L.) and a tree species (*Cassia fistula* L.) (Pandey, 2005). In general, the main factor inhibiting the growth of plants subject to the influence of atmospheric pollutants is the reduction of photosynthesis resulting from smaller leaf area and the concentration of photosynthetic pigments, as well as stomatal closure (Agrawal et al., 2003; Pandey, 2005).

Dry mass production in all organs was lower in plants exposed to the pollutant plume than in control plants. Leaves were the most negatively affected part of the plant (92.96% smaller), followed by roots (80.88%) and stems (66.95%) (Table 2). It is possible that the notorious decrease in dry mass production in all organs reflects the intense negative effect of the pollutant plume on the leaf area and the number of leaves.

In three locations of the industrial pole of Cubatão, São Paulo, Brazil, it was verified that SO_2 accumulation in the atmosphere negatively affected the dry mass production in all the organs of *Tibouchina pulchra* Cogn., especially in roots, reducing the root/shoot ratio (Szabo et al., 2003). Agrawal et al. (2003) observed that the accumulation of SO_2 , NO_2 and O_3 in peri-urban areas of Varanasi, India, may influence the productivity of some agricultural crops of *Vigna radiata*, *Beta vulgaris*, *Triticum aestivum* and *Brassica compestris*, in view of the effects of these pollutants on various physiological parameters of plants such as biomass, height, pigments and seed production.

Table 2. Leaf area, number of leaves, and dry mass of leaves, stems and roots of *Psidium cattleianum* plants grown in the control and exposed to the plume of pollutants from mining industrial activity at the end of the experimental period. Brumado, Bahia, Brazil.

Characteristics evaluated	Control	Plume
Leaf area (cm ²)	2300.00 a	200.00 b
Number of leaves	326.31 a	49.31 b
Dry mass of leaves (g)	43.62 a	3.07 b
Dry mass of stems (g)	37.74 a	12.47 b
Dry mass of roots (g)	38.39 a	7.34 b

Means followed by the same letters, in the same line, indicate absence of significant differences, according to the t test ($p < 0.05$).

The percentage of leaf necrosis was significantly higher in plants exposed to the pollutant plume (2.84%) than in control plants (0.74%) (Figure 2). The symptoms manifested as dark spots of different sizes, randomly distributed in the leaf blade (Figure 3A).

Leaf necrosis is an injury commonly found in plants subject to environmental contamination whose occurrence is attributed to several pollutants such as fluorine and sulfur oxides (Moraes et al., 2002; Agrawal et al., 2003). In the industrial pole of Cubatão, São Paulo, Brazil, symptoms of leaf necrosis caused by air pollutants were more easily seen in *P. cattleianum* than in *P. guajava* (Moraes et al., 2002). *Prosopis cineraria*, *Azadirachta indica* and *Phoenix dactilifera* plants around a refinery in Muscat (Oman) showed typical symptoms of necrosis due to exposure to SO₂ and other polluting gases (Abdul-Wahab & Yaghi, 2004). In general, the effects of SO₂ that cause leaf injury are influenced by biological factors such as inherent characteristics of the species and by the association of this gas with other pollutants, such as NO₂ (Abdul-Wahab & Yaghi 2004; Bulbovas et al. 2015).

The SPAD index was lower in the plants exposed to the pollutant plume than in the control plants, and this difference was more significant in the winter than in the summer (Table 3).

Symptoms of chlorosis, characterized by yellowing between leaf ridges, reinforce the visible effects of the pollutant plume on chlorophyll content (Figure 3B). Chlorophyll extracts showed that the SPAD index in the plants exposed to the pollutant plume should be especially attributed to the lower chlorophyll a content, considering that there was no difference of chlorophyll b content between treatments (Figure 4).

Changes in chlorophyll content in plants exposed to the influence of atmospheric pollutants have been well reported in the literature. A reduction in the levels of chlorophyll a and b (Chauan, 2010) was reported in *Religious ficus* submitted to high concentrations of NO_x, SO₂ and particulate material. Leaves of *Azadirachta indicata*, *Nerium oleander*, *Mangifera indica* and *Dalbergia sissoo* submitted to polluted

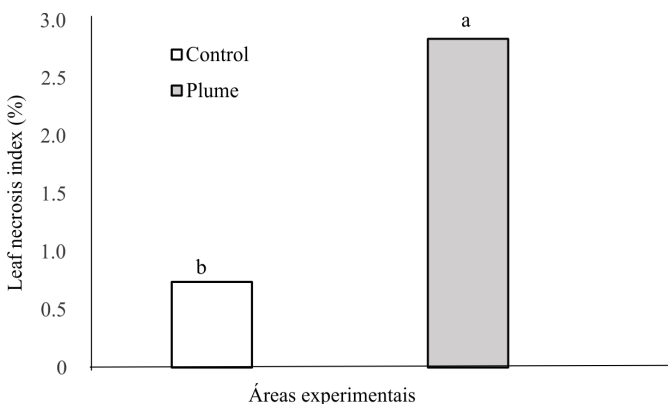


Figure 2. Leaf necrosis index in *Psidium cattleianum* plants grown in the control area and under exposure to pollutants released from industrial mining activity at the end of the experimental period. Brumado, Bahia, Brazil.

A.



B.



Figure 3. Symptoms of necrosis (A) and chlorosis (B) in leaves of *Psidium cattleianum* exposed to the plume of atmospheric pollutants. Brumado, Bahia, Brazil.

Table 3. SPAD index on leaves of *Psidium cattleianum* cultivated in the control area and under exposure to the plume of pollutants from mining industrial activity. The data refer to mean values of the evaluations performed in the summer and winter seasons, respectively. Brumado, Bahia, Brazil.

Evaluation moments	SPAD index	
	Control	Plume
Summer	55.14 a	49.98 b
Winter	53.96 a	36.69 b

Means followed by the same letters, in the same line, indicate absence of significant differences, according to the t test ($p < 0.05$).

environments also had significant reductions in chlorophyll content (Giri et al., 2013).

One of the factors that most contribute to the degradation of chlorophylls is pH (Streit et al., 2005). Acidification of plant tissues caused by some pollutants such as SO₂ (Rabe & Kreeb, 1980) and HNO₃ (Riddell et al., 2012) may lead to lower chlorophyll levels in plants, followed by formation of pheophytin, reducing the photosynthetic capacity of plants.

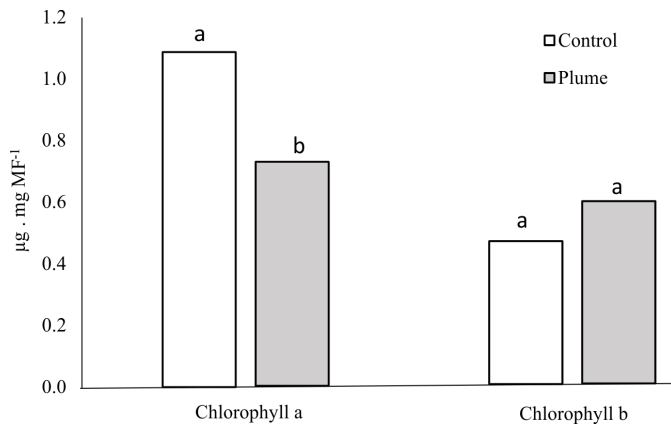


Figure 4. Chlorophyll a and b content of leaves of *Psidium cattleianum* cultivated in the control area and under exposure to the plume of pollutants from mining industrial activity at the end of the experimental period. Brumado, Bahia, Brazil. In each type of chlorophyll, the same letters indicate absence of significant difference, according to the t test ($p < 0.05$).

Production of phaeophytin is also related to the loss of green color in leaves (Streit et al., 2005). The degradation of chlorophyll a is faster than that of chlorophyll b (Rabe & Kreeb 1980), and their reduction may indicate low or moderate atmospheric pollution caused by SO_2 (Rabe & Kreeb, 1980). The determination of chlorophyll content is important to evaluate the effects of pollutants on the metabolism of plants, which may have direct effects on their growth.

The content of soluble sugars was not affected by exposure to the pollutant plume, either in the leaves or in the roots (Figure 5). Soluble sugars are an important constituent and source of energy for all living organisms, and their levels in leaf tissues should be analyzed as the result of the interaction between different physiological events. Thus, soluble sugar content alone may not be enough to establish a relationship between carbohydrate metabolism and characteristics associated with growth such as plant height, number of leaves, leaf area and dry mass of different parts of the plant. The trioses that are produced during photosynthesis may follow distinct metabolic pathways, such as starch synthesis, for storage purposes, or sucrose, which is exported to supply carbon and energy demands in the rest of the plant (Lunn & MacRae, 2013). In addition, a considerable fraction of soluble sugars is oxidized during respiration (Taiz et al., 2017).

Atmospheric pollutants can influence in different ways the main metabolic routes that determine the content of soluble sugars, such as primary photosynthesis, the synthesis and hydrolysis of starch and sucrose, and respiratory activity. Thus, besides being an indicator of the physiological activity of the plant, soluble sugar content determines the sensitivity of plants to air pollution (Rai, 2016).

There are controversial studies in the literature about the relationship between atmospheric pollutants and soluble sugars, suggesting that there is no standard behavior in

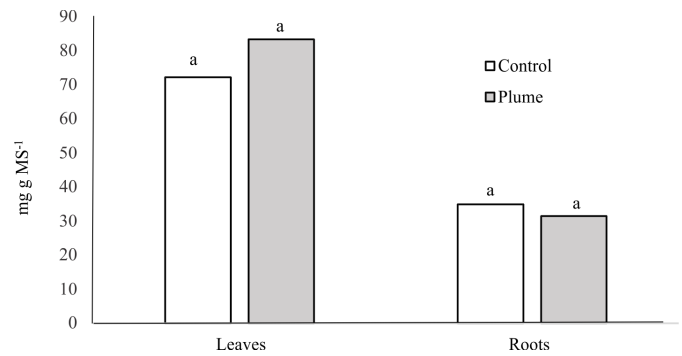


Figure 5. Soluble sugar content in leaves and roots of *Psidium cattleianum* cultivated in the control area and under exposure to the plume of pollutants from mining industrial activity at the end of the experimental period. Brumado, Bahia, Brazil. For each organ of the plant, the same letters indicate absence of significant difference, according to the t test ($p < 0.05$).

terms of metabolism of synthesis and degradation of soluble sugars in response to this kind of environmental stress. Some authors state that high concentrations of SO_2 and NO_2 in the atmosphere may cause a decrease in the content of soluble sugars in leaves for varying reasons. The possible increase in respiratory rate, for example, may be associated with higher energy consumption, under stressful conditions, reducing the content of soluble sugars in leaves (Lorenc-Plucinska, 1982). In strawberry plants, gases such as CO , NO_x and SO_2 stimulate the production of oxygen reactive species, resulting in lowering the soluble sugar content due to photosynthesis inhibition (Muneer et al., 2014). On the other hand, Seyyednejad et al. (2009) found higher soluble sugar content in leaves of *Callistemon citrinus* exposed to atmospheric pollution, whose increase was attributed to the possible existence of some protective mechanism.

Conclusion

The species *P. cattleianum* is sensitive to atmospheric emissions of SO_2 and NO_x .

In the plants exposed to the pollutant plume, inhibition in the growth in terms of height and size and quantity of leaves was observed, besides symptoms of chlorosis and necrosis. Dry matter production was also lower in all organs.

Chlorophyll a content was lower, but chlorophyll b did not change.

Soluble sugar content was not affected by the pollutants.

Atmospheric emissions from the mining industry negatively affect the morphophysiological characteristics of *P. cattleianum*, inhibiting plant growth.

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