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Impacts of urban environment on the leaves of two popular medicinal plants: *Lippia alba* (Mill.) N. E. Brown (Verbenaceae) and *Coleus barbatus* (Andrews) Benth. (Lamiaceae)

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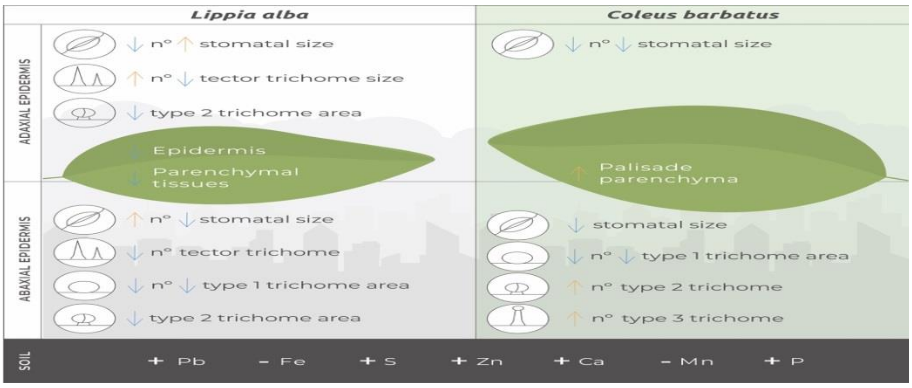
Abstract: Previous studies have demonstrated that urban environmental conditions are sufficient to induce changes in leaf structure and alter the toxicological properties of leaf extracts from medicinal plants. The present study aimed to detect changes in the structure of leaves of *Lippia alba* (Mill.) N. E. Brown (Verbenaceae) and *Coleus barbatus* (Andrews) Benth. (Lamiaceae) induced by interactions with pollutants and abiotic factors in an urban environment. Control areas were used to compare the observed results. Leaves were processed according to standard techniques for plant anatomy, and a chemical analysis of the soil in the different study sites. For the external surface of the leaves, differences were observed in the stomatal density and the structure and density of tector and glandular trichomes. This study highlights the sensitivity/plasticity of secretory structures in a comparative way. The higher concentration of lead in the soil, the high levels of O_3, high temperature, and lower levels of rainfall observed in the urban site may have influenced the variation in these anatomical characteristics. These aspects are essential for establishing best practice guidelines for using species for medicinal purposes.

Graphic Abstract – Scheme comparing the structural differences found in the two study species (*Lippia alba* and *Coleus barbatus*) developing in the urban site, highlighting the soil conditions of this site.



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Highlights

• This study highlights the sensitivity/plasticity of external secretory structures to urban environmental conditions in a comparative way.

• Differences were observed in the density of stomata and in the structure and density of tector and glandular trichomes.

• The location of the secretory structure is important in plants in polluted environments.

Keywords: Cidreira; False boldo; Bioindicators; Anatomical characteristics.

Introduction

Medicinal plants are widely used by Brazilians (Saúde, 2012; IBGE, 2014). Despite the intense urbanization in recent years, Brazilians continue to grow plants in home gardens to use them to prevent and treat diseases (Botelho *et al.*, 2014). In Rio de Janeiro, the second largest city in Brazil and the fourth largest city in Latin America, most of the urban population obtains medicinal plants by growing them and sharing what they grow (Silva *et al.*, 2014; Sartori *et al.*, 2019). However, the climate and pollution of large cities can affect the quality and safety of medicinal plants, affecting their therapeutic efficacy. It is essential to monitor air and water quality and take steps to minimize the exposure of medicinal plants to pollutants in urban areas (Christo *et al.*, 2010; Masson *et al.*, 2020). In the center of large metropolises, such as Rio de Janeiro, the local climate is influenced by urban development through warming, which is known as the heat island effect (Masson *et al.*, 2020). The construction of buildings influences the wind and temperature of cities, modifying the direction of storms and precipitation patterns (Masson *et al.*, 2020). Furthermore, urbanization is almost always accompanied by air pollution, soil contamination, and changes in carbon emission levels, among other environmental problems (Gurjar *et al.*, 2008; Roy *et al.*, 2012).

Plant responses to pollutants can be observed at biochemical, microscopic, or macroscopic levels (Khosropour *et al.*, 2019; Çali & Karavin, 2020; Guerrero *et al.*, 2020; Petrova, 2020). In Brazil, several studies have demonstrated the adverse effects of pollutants on plants through the passive biomonitoring of organisms in polluted areas (Silva *et al.*,, 2017; Vasconcellos *et al.*, 2017; Lüttge & Buckeridge, 2020; Vasconcellos & Callad, 2020; Silva *et al.*, 2021). Analyzing medicinal plants in the city of Rio de Janeiro has been enlightening in relation to the impact of urban conditions on the structure and chemistry of leaves and on the cytotoxicity of extracts (Bezerra *et al.*, 2020, 2021). The different levels of structural change can define the degrees of tolerance of a plant to a given stress (Grantz *et al.*, 2003; Guerrero *et al.*, 2020). Therefore, a comparison of anatomical trait profiles can reveal differences in the ability of plants to cope with urban environmental conditions and resources under different forms of growth (Guerrero *et al.*, 2020).

Ethnobotanical studies in Rio de Janeiro revealed that Lippia alba (Mill.) N. E. Brown (Verbenaceae) and Coleus barbatus (Andrews) Benth (Lamiaceae) are among the ten most used medicinal plants by the population (Christo *et al.*,, 2010; Bochner *et al.*,, 2012; Silva *et al.*,, 2014). Based on this, L. alba and C. barbatus were selected for the study. These species are listed as widely used medicinal plants by Brazilians (Lorenzi *et al.*, 2021). Lippia alba is native to Brazil and popularly known as erva-cidreira (Lorenzi *et al.*, 2021). Its leaves are used because of their antispasmodic, stomachic, soothing, and digestive properties, as well as for insomnia and asthma, and for oral diseases due to their bactericidal, antiseptic, and astringent properties (Oliveira *et al.*, 2006). Lippia alba is the most studied species of Lippia and exhibits high phenotypic and genomic plasticity (Torres & Lopez, 2007; Reis *et al.*, 2014).

Coleus barbatus is a cultivated species known as falso-boldo or boldo-brasileiro (Lorenzi *et al.*,, 2021). It has various uses in traditional Hindu, Ayurvedic medicine and folk medicine in Brazil, tropical Africa, and China (Alasbahi & Melzig, 2010). This species is traditionally used in folk medicine to treat health problems, such as digestive disorders, hepatitis, respiratory disorders, heart disease, and certain nervous system disorders (Lukhoba *et al.*, 2006; Alasbahi & Melzig, 2010). In addition, C. barbatus is one of the species on the National List of Medicinal Plants of Interest to the Unified Health System (RENISUS) that was implemented by the Brazilian Ministry of Health in 2008 (Saúde, 2009). Although people commonly grow these plants in urban areas for consumption, it is still unknown how these plants are affected by environmental conditions and to what extent this can cause side effects when they are used as herbal medicines.

The secretory structures and secondary metabolites of these plants are directly related to their medicinal properties (Okem *et al.*, 2015). In previous studies, changes induced by exposure to an urban environment were observed in the leaf structure of Eugenia uniflora L. (Myrtaceae) (Bezerra *et al.,* 2020). One response observed was an increase in the density of defense structures, which are the internal secretory glands in E. uniflora. Furthermore, these developmental conditions were sufficient to alter the toxicological potential of an alcoholic extract of the leaves of this species (Bezerra *et al.,* 2021). After these observations were made, a question arose about what would be found if comparative studies were made with species with external secretory structures and different habits in the same sites where E. uniflora was studied. The species chosen for this study allowed us to investigate the sensitivity/plasticity of these external secretory structures to urban environmental conditions in a comparative way.

Therefore, this study aimed to investigate possible variations in the external and internal structure of leaves of L. alba and C. barbatus, grown in the city of Rio de Janeiro, and to detect possible changes directly related to exposure to this urban environment.Materials Topographic sheets from the Survey of India were used to create the base map of the research region (Madurai South Taluk), which was then digitalized using ArcGIS 10.2 software (Figure1). Between, 2008,2022, fourteen groundwater samples (bore and open wells) were taken from the research region before and after the monsoon season. The Water Resources Department (WRD) noted each sample station's position coordinates using a handheld GPS. For the research period of 2008 to 2022, the Chief Engineer, WRD, State Ground and Surface Water Resources Data Centre, Taramani, Chennai, provided the test findings for the samples.

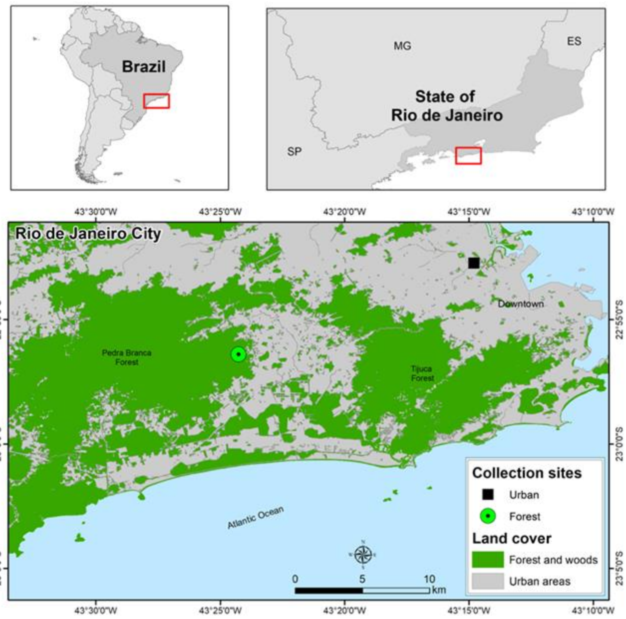

Figure 1: Map highlighting study sites in the city of Rio de Janeiro, Brazil. Fonte: Bezerra *et al*., 2020.

Material and methods

Study sites and collection conditions

The study was carried out at two sites in the Atlantic Forest domain, in Rio de Janeiro/RJ city, Brazil: (1) urban site located at Fundação Oswaldo Cruz Manguinhos, on the side of the main urban road on the city, Avenida Brasil. In this urban road, the action of various pollutants, cytotoxic and mutagens were observed (INEA 2015, 2020; Rainho *et al.,* 2013) and (2) forest site, our control area, located at Fundação Oswaldo Cruz Mata Atlântica, on the edge of Pedra Branca State Park (Figure 1). In the urban site, the specimens were cultivated in the garden of the Fiocruz Manguinhos Campus and the forest site; the species were cultivated in the horto of the Fiocruz Mata Atlântica Campus. The soil surface on both sites presented litter.

Leaf sampling was carried out in 2018 at both sites, on consecutive days, at 9 am, according the following sampling criteria: date of collection in a week with sunny days; selection of 5 individuals of Lippia alba and Coleus barbatus at each study site; healthy leaves, exposed to the sun and located at the third node from the apex.

The climatic data of each site were obtained from the meteorological stations of the Alerta Rio System, of the City Hall of Rio de Janeiro closest to each of the study sites: São Cristóvão Meteorological Station for the urban site (-22.981289-43, 405075) and Rio Centro Meteorological Station for the forest site (-22.981289 -43.405075). Table 1 shows the environmental characteristics of the forest and urban sites. Air quality data were obtained from the website of the Instituto Estadual do Ambiente (INEA).

Table 1: Environmental characteristics of forest and urban locations along with climatic variables during the study period.

	Forest	Urban
Geographic location	(-22.939889, -43.404424)	(-22.878639, -43.246621)
Altitude (m.a.s.l)	24	30
Accumulated annual precipitation (mm)	1523.8	1088.8
Relative air humidity - annual average (%)	82	72
Average temperature - annual (°C)	24	24.5
Topsoil cover	burlap	burlap

X-Ray Fluorescence (XRF)

Chemical elemental analysis of soil samples from the two study sites was performed using X-ray fluorescence (XRF) analysis. Five soil samples at five sampling points were collected at a depth of approximately 10 cm, totaling 25 samples at each study site. The litter layer was removed before soil collection. Soil sampling points were close to the specimens evaluated in the study. Soil samples were homogenized in the laboratory using the row technique (Horwitz, 1990).

The samples were dried in an oven at approximately 60 ºC (48h). Then, the dried samples were mechanically ground using an agate mill and sieved with nylon mesh (75 µm). Aliquots of 500 mg of each sample were compacted at a pressure of 2.32×108 Pa for approximately 15 minutes to obtain thin pellets with a diameter of 2.54 cm and a surface density of 100 mg/cm^2 (Anjos *et al.*, 2000; Santos *et al.*, 2019).

XRF analysis of the pellets were performed using the Epsilon 1 equipment (Malvern Panalytical) with the Silver anode (Ag) and SDD detector (Silicon Drift Detector) (energy resolution <135 eV for Mn-Kα). Two different experimental conditions were used in the analysis of the samples. The first experimental condition was used for a better excitation of the low Z elements, using 10 kV, 336 µA, and an acquisition time of 600s. The second experimental condition for excitation of high Z elements using 50 kV, 100 µA, 300 s and a Cu filter (500 µm). Quantitative analysis were performed using the Epsilon 1 software.

The precision and accuracy of the XRF system were performed using the measurements of the certified sample BCS-CRM-353. The relative errors found were less than 23%. The lower atomic number of elements presented a higher relative error due to the difficulty of analyzing light elements in experiments carried out at room temperature and, therefore, were disregarded.

Anatomical Analysis

For the characterization of the epidermis in surface view, samples from the middle region of the leaves of the third node were fixed in an aqueous solution of 2.5% glutaraldehyde, 4.0% formaldehyde, and sodium cacodylate buffer at 0.05 M and pH 7.2 (Da Cunha, M., 2000), dehydrated in ascending alcoholic series (Johansen, 1940) and submitted to the critical point of liquid CO2, with the aid of the Bal-Tec Critical Point Dryer CPD 030 device with carbon tape on proper supports and covered with a thin layer of 20 nm gold (Bal-Tec Sputter Coater SCD 050). The images were obtained using a scanning electron microscope (SEM) (I think it was the Jeol - Zeiss EVO 40 (Germany) SEM) at a voltage of 15 kV.

For anatomical analysis, samples from the middle region of the leaves of the third node were fixed as previously described, washed in 0.05 M cacodylate buffer pH 7.2, and post-fixed in 1% osmium tetroxide. The samples were then dehydrated in a series of acetone graders. Subsequently, the material was infiltrated with Epon® synthetic resin. The samples were sectioned using a rotating microtome at 5 µm thickness. Histological sections were stained with Toluidine blue O (O'Brien *et al.*, 1964), mounted on Entellan®, and observed under light microscopy. The analysis was performed with a light microscope Olympus BX 41, and the images were obtained with a Q Collor R3 video camera attached to the same microscope.

The following leaf parameters were evaluated: the thickness of the epidermis on the adaxial and abaxial surfaces (in µm), the palisade parenchyma (in µm), the spongy parenchyma (in µm) and the mesophyll (in µm); length of stomatal cells and tector trichomes (in µm); frequency/mm2 of stomata and tector and glandular trichomes; and area of glandular trichomes (in µm). Twenty-five fields were examined for each sheet investigated. Analysis were performed using the Image-Pro Express 6.0 digital image processing system.

Statistical analysis

All quantitative results were statistically analyzed using the STATISTICA 7.0 software (StatSoft, Inc., USA). Data were tested for normality and homoscedasticity using the Shapiro-Wilk and Levene tests, respectively. The comparison of the results obtained for the two sites was made, for parametric data, using Student's t test and for non-parametric data, using the Mann-Whitney test, at a significance level of 95% (Zar, 2010).

Results

The elemental composition of the soil close to the individuals of each species studied had a similar chemical profile. In the soil samples from the two investigated sites, 20 chemical elements were detected: aluminum (Al), silicon (Si), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), titanium (Ti), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), bromine (Br), rubidium (Rb), strontium (Sr), zirconium (Zr), and lead (Pb). Table 2 provides the concentrations of all elements found in these samples.

Regarding the homogenized soil sample close to individuals of Lippia alba, Si, Cl, Cr, Cu, Ga, Br, and Sr did not differ statistically between the two sites. On the other hand, Al, Ti, Mn, Fe, Ni, and Rb had higher concentrations in soil samples from the forest site, while P, S, K, Ca, Zn, and Zr had higher concentrations in the urban site.

Table 2: Elemental concentrations in soil samples collected near *Lippia alba* and *Coleus barbatus* plant at urban and forest sites, along with results from Student's t-test and Mann–Whitney U test.

	Lippia alba					***Coleus barbatus***			
Elements	**Forest**	**Urban**	**t value**	**U**	**p**	**Forest**	**Urban**	**t value**	**p**
Al (%)	13.315	6.479	34.5764		<<0.05*	12.636	11.255	28.6093	<<0.05*
Si (%)	27.597	25.445		0.0	0.05	26.047	22.712	11.8304	<<0.05*
P (ug/g)	3960	6710	-7.9010		<<0.05*	3970	6780	-14.7482	<<0.05*
S (ug/g)	620	3090	-14.4293		<<0.05*	980	3000	-9.3569	<<0.05*
Cl (ug/g)	2040	2310	-0.3311		0.76	2130	2110	-0.0588	0.96
K (%)	3.981	4.276	-4.2257		0.01*	4.1	2.305	27.3308	<<0.05*
Ca (%)	0.992	5.903	-17.2898		<<0.05*	1.53	5.696	-30.1604	<<0.05*
Ti (%)	1.545	0.727	18.8904		<<0.05*	1.63	1.177	18.9924	<<0.05*
Cr (ug/g)	100	90	0.2132		0.84	100	169.5	-12.6568	<<0.05*
Mn (ug/g)	2470	690	14.7009		<<0.05*	1930	809.5	109.9938	<<0.05*
Fe (%)	11.356	4.347	28.2494		<<0.05*	11.372	8.87	15.0764	<<0.05*
Ni (ug/g)	110	60	12.0208		<<0.05*	118.7	90	6.6086	<<0.05*
Cu (ug/g)	120	150		0.0	0.05	118.2	218.5	-5.9333	<<0.05*
Zn (ug/g)	330	480	-6.1713		<<0.05*	325.4	643.1	-9.9603	<<0.05*
Ga (ug/g)	70	40		0.0	0.05	80	61.6	7.1533	<<0.05*
Br (ug/g)	20	30		1.5	0.19	40	42.1	-1.6811	0.17
Rb (ug/g)	390	200	14.9666		<<0.05*	430	155.2	20.5731	<<0.05*
Sr (ug/g)	240	320	-6.1470		<<0.05	290	315.7	-1.0268	0.36
Zr (ug/g)	610	740		0.0	0.05*	823.1	849.5	-0.5747	0.60
Pb (ug/g)	100	180	-4.8020		0.01*	100	170	-5.6856	<<0.05*

* It represents a significant difference with a 95% confidence interval (p). Values referring to the average value (M).

Regarding the homogenized soil sample close to individuals of Coleus barbatus, Cl, Br, Sr, and Zr did not differ statistically between the two sites. Like the results for the soil close to the previous species, some chemical elements varied between the investigated sites. The elements Al, Si, K, Ti, Mn, Fe, Ni, Ga, and Rb had higher concentrations in soil samples from the forest site, while P, S, Ca, Cr, Cu, Zn, and Pb had higher concentrations in the urban site.

Lippia alba leaves have a uniseriate epidermis, anomocytic stomata with a large substomatic chamber located on both sides, dorsiventral mesophyll, and tector and glandular trichomes. The tector trichomes are uniseriate, multicellular, pointed, and erect. There are two types of glandular trichomes: type 1, which is formed by a narrow basal cell and a globose capitate portion; and type 2, which is formed by a basal cell, an intermediate cell, and a bicellular capitate portion. The type 1 trichomes are only present on the abaxial surface. The type 2 trichomes are on the adaxial and abaxial surfaces.

Coleus barbatus has leaves with a uniseriate epidermis covered by a thin cuticle, anomocytic stomata on the adaxial and abaxial surfaces, tector and glandular trichomes, and dorsiventral mesophyll. The tector trichomes are uniseriate, multicellular, pointed, erect or slanted, and have many cells at the base. There are three types of glandular trichomes: type 1 - glandular trichome, which has a basal cell (epidermal) and a globose capitate portion; type 2 - short-capitate glandular trichome, which has one or two basal cells, one or two peduncular cells, and one or two apical secretory cells; and type 3 - long-capitate glandular trichome, which has two or more basal cells, two or three peduncular cells, and one apical secretory cell.

The quantitative parameters measured for L. alba and C. barbatus in the forest and urban sites and the results of the statistical tests are provided in Table 3. All parameters referring to the mesophyll of L. alba were statistically different between the two sites. In general, the leaf blade of the samples from the urban site presented a thinner mesophyll layer, associated with a laxer spongy parenchyma and a larger and deeper substomatic chamber (Figure 2B). The comparison of

Table 3: Quantitative leaf parameters of *Lippia alba* and *Coleus barbatus* measured at forest and urban sites, along with the results of Student's t-test and Mann–Whitney U test.

Lippia alba					
Parameters	**Forest** (M)	**Urban** (M)	**t**	**U**	**P**
Adaxial					
GT type 2 (nº)	1.8	2.1	-0.7		0.49
GT type 2 (área/µm)	855.25	616.9	2.8		0.04*
GT type 3 (nº)	0.9	0.8	0.4		0.67
TT (nº)	11.6	14.7		0.0	0.04*
TT (µm)	136.6	99	3.1		0.04*
Stomata (nº)	26.2	1.5		0.0	0.03*
Stomata (µm)	20.3	22	-3.5		0.03*
Abaxial					
GT type 1 (nº)	9.1	2.1	6.5		<0.01*
GT type 1 (área/µm)	2555.9	1171.4		0.0	0.02*
GT type 2 (nº)	2.3	0.7	2.3		0.05
GT type 2 (área/µm)	563.5	354.3		1.0	0.04*
TT (nº)	27.5	18.5	4.0		<0.01*
TT (µm)	164.2	101.1	1.2		0.28
Stomata (nº)	43.4	62.2	-3.5		0.03*
Stomata (µm)	24.0	14.2	3.9		<0.01*
Leaf Blade (µm)					
Mesophyll	189.79	104.05	3.9		<0.01*
Palisade Parenchyma	96.27	51.58	2.9		0.02*
Spongy Parenchyma	95.71	57.19	5.0		0.002*
Adaxial Epidermis	29.94	20.82	6.6		<<0.05*
Abaxial Epidermis	17.76	13.81	4.1		<0.01*

Coleus barbatus					
Parameters	**Forest** (M)	**Urban** (M)	**t**	**U**	**P**
Adaxial					
GT type 1 (nº)	0.14	0.14	-0.99		1.00
GT type 1 (área/µm)	7887	8269		0.0	1.00
GT type 2 (nº)	10.5	10.4	0.00		0.85
GT type 3 (nº)	1.0	1.0	-0.19		1.00
TT (nº)	8.8	8.1	0.00		0.34
Stomata (nº)	8.2	0.7		0.0	<0.01*
Stomata (µm)	36.1	21.8		0.0	<0.01*
Abaxial					
GT type 1 (nº)	6.6	4.7	-3.29		<<0.05*
GT type 1 (área/µm)	10520	9442	-2.72		<0.01*
GT type 2 (nº)	0.5	1.7	3.12		<<0.05*
GT type 3 (nº)	2.25	4.81	3.25		<0.01*
TT (nº)	11.8	12.1	0.34		0.73
Stomata (nº)	50	53	0.70		0.48
Stomata (µm)	41.47	36.1	-3.24		<<0.05*
Leaf Blade (µm)					
Mesophyll	159.03	176.66	-2.09		0.09
Palisade Parenchyma	96.34	127.49	-3.81		<0.01*
Spongy Parenchyma	60.75	59.14	-0.40		0.70
Adaxial Epidermis	22.98	24.57	-0.72		0.50
Abaxial Epidermis	12.61	14.08		3.0	0.28

* It represents a significant difference with a 95% confidence interval (p). Values referring to the average value (M). Abbreviations: GT- glandular trichome; TT- tector trichomes.

the external structure of the leaves of L. alba showed that there are differences regarding the density of (1) stomata, (2) tector trichomes, and (3) type 1 trichomes between the leaves of the two sites (Table 3; Figure 2 C-F). Regarding the dimension (area/µm), these attributes differed in terms of (1) stomata, (2) tector trichomes, and (3) type 1 and 2 trichomes. The leaves that developed in the urban site have a lower density of stomata on the adaxial surface, which are larger than those observed in the control (the forest site – Table 3). On the other hand, the tector trichomes are denser and smaller. Although the tector trichomes on the adaxial surface of the leaves in the urban site are mostly smaller, some isolated trichomes are much larger than the average (Figure 2D). For the samples from the urban site, the type 2 glandular trichomes had a smaller area of the structure. On the abaxial surface of the leaves from the urban site, smaller stomata at a greater density were observed, which is the opposite of what was observed on the adaxial surface of the leaves from the same site. Regarding the trichomes, a lower density of type 1 tector and glandular trichomes and a smaller area of type 1 and 2 glandular trichomes were observed (Figure 2E-F).

Of all the parameters of the leaf blade analyzed for *C. barbatus*, only the palisade parenchyma differed statistically between the two study sites (Table 3; Fig 3A-B). The comparison of the external structure of the leaves of *C. barbatus* showed that there are differences in the density and size of (1) stomata and (2) glandular trichomes between the leaves of the two sites (Fig. 3 C–F). The leaves that developed in the urban site had a lower density of stomata on the adaxial surface that were smaller than those observed in the forest site. A reduction in stomata size was also observed on the abaxial surface of leaves from the urban site. On the abaxial surface of the leaves from the urban site, the type 2 and 3 trichomes were more numerous, and the

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type 1 glandular trichomes were less numerous and had a reduced area.

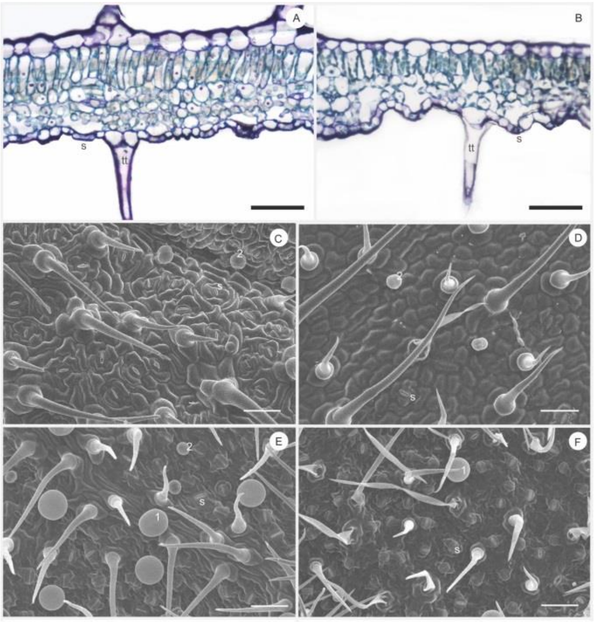

Figure 2: *Lippia alba* leaf. (A; B) - Cross section of the leaf blade of individuals from forest (A) and urban (B) sites. (C-F) - Adaxial (C; D) and abaxial (E, F) epidermis, from leaves from forest (C, E) and urban (D, F) sites. s: stomata; tt: tector trichomes; 1: type 1 glandular trichomes; 2: type 2 glandular trichomes. (A, C, E – forest site) (B, D, F – urban site); (A; B- MO) (C, D, E, F – SEM). Bar = 50µm.

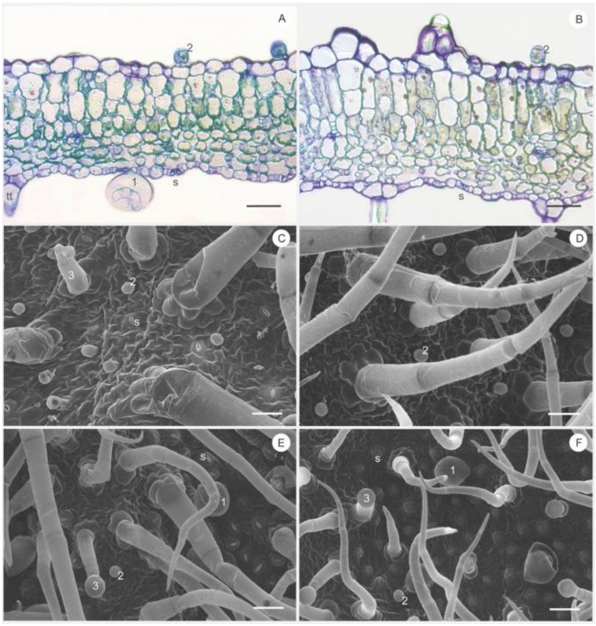

Figure 3: *Coleus barbatus* leaf. (A; B) - Cross section of the leaf blade of individuals from forest (A) and urban (B) sites. (C-F) - Adaxial (C; D) and abaxial (E, F) epidermis, from leaves from forest (C, E) and urban (D, F) sites. s: stomata; tt: tector trichomes; 1: type 1 glandular trichomes; 2: type 2 glandular trichomes; 3: type 3 glandular trichomes. (A, C, E – forest site) (B, D, F – urban site); (A; B- MO) (C, D, E, F – SEM). Bar = 50µm.

Discussion

The urban site examined in this study experiences elevated temperatures. In contrast, in the forest site the relative humidity and rainfall indices are higher than those observed in the urban site, especially during the rainy season, which coincides with the hottest period of the year in the study region. Thus, these differences in temperature, precipitation, and air humidity are expected to directly affect the leaf structures of plants in this location (Kardel *et al*., 2010; Karabourniotis *et al*., 2020).

Structural analyses can help reveal the impact of urban environments on plants, making it possible to identify different adaptive strategies and degrees of plant tolerance (Grantz *et al*., 2003; Sant'anna-Santos *et al*., 2012). Previously, we hypothesized that a plant grown in an urban environment, with the climatic characteristics described in this study, would invest in an abundance of palisade parenchyma and tector trichomes as a protective barrier to excessive evapotranspiration by the stomata. In addition, a greater investment in glandular trichomes was hypothesized due to an increased demand for secondary metabolites as a response to protect the plant from the less favorable environment (Pérez-Estrada *et al*., 2000). Our observations reflect a less predictable scenario for *L. alba*, with a reduced number of tector trichomes and a lower abundance of palisade parenchyma. In the urban site, the leaves of *C. barbatus* showed a significant decrease in stomatal density on the adaxial surface, an increase in stomata size and a significant increase in palisade parenchyma, which is possibly an attempt to tolerate the adverse effects of pollutants (Khosropour *et al*., 2019).

In drier climates, which often occur in urban environments, choosing between carbon gain and saving water requires appropriate structural and metabolic modulations (Karabourniotis *et al*., 2014). In *L. alba*, a decrease in the growth of photosynthetic tissues was observed in the leaves from the urban site. Stressful conditions in this site may have led to a lower investment in chlorophyll parenchyma. Low photosynthetic capacity and growth capacity are related to biochemical constraints and structural limitations of the leaf, such as smaller stomata or lower stomatal density and high leaf mass per area, leading to reduced mesophyll conductance (Hikosaka 2010).

Under the urban site conditions, stomata exhibited plasticity (Bezerra *et al*., 2020), which is an essential attribute in environmental biomonitoring (Balasooriya *et al*., 2009; Kardel *et al*., 2010; Wuytack *et al*., 2010). These modifications improve the control of gas exchange and, consequently, the entry of pollutants through stomata (Kardel *et al*., 2010). In this study, the stomatal frequency significantly differed between *L. alba* and *C. barbatus* specimens in both sites. Both species had a reduction in stomatal density on the adaxial surface. The data indicate a strategy of concentrating stomata on the abaxial surface of the leaf of the plants in the urban site, which may be related to an attempt to reduce water loss by investing in less exposed stomata (Karabourniotis *et al*., 2020). These observations may indicate unfavorable conditions for the normal physiological development of the plant, considering that a change in the number of stomata is common under adverse conditions. Changes in stomatal density, distribution, and morphology are essential characteristics in adaptation/tolerance to air pollution (Rashidi *et al*., 2012).

In addition to temperature and atmospheric pollutants, the urban environment has several factors that can interfere with the development of plants, such as the presence of contaminants in the soil (Masson *et al*., 2020). The soils of the two studied sites have statistical differences for most elements, such as S, Ca and Pb in the urban site and Mn and Fe in the forest site. Calcium is commonly found in particulate material emitted into the atmosphere by construction sites (Resende 2007), a common practice in the region where the urban site is located.

Soil and water contamination by lead (Pb) is increasing every day due to several activities linked to industrializsation and urbanization (Cândido *et al*., 2020). Atmospheric deposition of Pb constitutes one of the main absorption pathways for plants (Tomaševič & Anicic 2010; Hrotkó 2020). Pb is a non-essential toxic metal to plants and has no role in the plant cellular metabolism process. However, it is easily absorbed and accumulates in different parts of plants (Nas & Ali 2018). The results of this study indicate a higher concentration of this element in the urban site soil than in the forest site soil. In addition to the observed interferences in stomatal development, Koul & Bhatnagar (2017) found that Pb-treated plants also exhibited reduced trichome density on their leaves. These data suggest that the Pb concentration observed in the soil of the urban site may have interfered with the difference in the density of trichomes observed on the plants of the present study.

The structure and chemical constituents of trichomes can change due to factors such as radiation level, herbivory, water stress, salinity, and heavy metals (Liu *et al*., 2017; Karabourniotis *et al*., 2020). In addition to indicating how a plant interacts with the environment, serving as a bioindicator character, several studies have shown that various plant leaf trichomes participate in detoxifying toxic metals (Broadhurst *et al*., 2004; Domínguez-Solís *et al*., 2004). These epidermal leaf appendages can interact with atmospheric pollutants, such as ozone (O_3). Air quality data from the urban site showed high mean concentrations of O_3 (INEA, 2020). High tropospheric O_3 levels negatively affect plant growth and development (Li *et al*., 2018). Studies suggest that glandular trichomes are a chemical barrier that reduces O_3 uptake by the leaf, thus reducing the toxicity (Jud *et al*., 2016; Li *et al*., 2018; Oksanen 2018), and species with a lower density of glandular trichomes were more sensitive and vulnerable to O_3 stress than species with a high density of glandular trichomes. These results demonstrate that glandular trichomes on the leaf surface are essential to reduce toxicity by functioning as a chemical barrier that neutralizes O_3 before it enters the leaf (Li *et al*., 2018). This aspect may have induced the compaction of the leaf in *L. alba*, considering that the reduction in trichome density is probably related to the increased vulnerability of the leaf to this type of atmospheric pollutant.

More abundant tector trichomes facilitate the deposition of contaminants on the leaves (Hu *et al*., 2019; Howsam *et al*., 2000). On the other hand, dense layers of trichomes can prevent water loss directly by influencing the resistance to water vapor diffusion across the leaf surface or indirectly by regulating the energy balance and reducing the leaf blade temperature (Pshenichnikova *et al*., 2019; Karabourniotis *et al*., 2020). However, plants do not always increase the density of these structures in response to lower water availability.

This study points out a relationship between the density of stomata and tector trichomes on the epidermis of *L. alba* from the urban site. The adaxial surface had fewer stomata but an increased number of tector trichomes. However, on the abaxial surface, the number of stomata increased while the number of tector trichomes decreased. The ratio of trichomes to stomata was positively correlated in water stress experiments, indicating that these structures play an essential role in drought tolerance (Galdon-Armero *et al*., 2018). Due to a combination of evolutionary and ecological pressures, the characteristics of some species tend to vary in tandem, revealing a trade-off to increase fitness under certain environmental conditions (Klingenberg 2014; Messier *et al*., 2017).

In this sense, *C. barbatus* showed less sensitivity to the conditions in the urban site. The higher density of some types of glandular trichomes may have contributed to plant resistance, favoring the development of photosynthetic tissues. This difference may be due to the greater phenotype plasticity of exotic species, allowing them to colonize a broader range of environmental conditions (Guerrero *et al*., 2020). Glandular trichomes are generally the sites of secondary metabolite biosynthesis, and variation in their abundance is directly related to the production of constituent metabolites (Yadav *et al*., 2014). A decline in the density, length, and area of glandular trichomes on the leaves of *Artemisia annua* L. (Asteraceae) under water stress implied its direct effect on artemisinin and essential oil biosynthesis since these secondary metabolites are synthesized in glandular trichomes (Yadav *et al*., 2014). These results are notable because they demonstrate that the significant difference in the density and area of these secretory structures will directly interfere with secondary metabolites and, consequently, the popular use of this plant.

Our study found differences in the morphoanatomical properties of *L. alba* and *C. barbatus* growing in an urban environment. It suggests that *L. alba* and *C. barbatus* change their morphoanatomical properties when they live in an urban environment. Stressful conditions in this site may have influenced a lower investment in chlorophyll parenchyma abundance in *L. alba*. The higher density of some types of glandular trichomes may have contributed to the resistance of *C. barbatus* under the urban site conditions, favoring an increase in photosynthetic tissues. The higher concentration of lead in the soil, high levels of O_3, atmospheric pollution, high temperature, and lower levels of rainfall observed in the urban site may have influenced the variation in anatomical characters. Attributes analyzed, such as the variation in the number of stomata, tector trichomes and glandular trichomes, may have contributed to the adaptation of *L. alba* and *C. barbatus* specimens under the urban conditions and could possibly be used for biomonitoring in urban environments. More comprehensive studies are needed about the interference of urban environments on the metabolites produced by secretory structures and, consequently, on their biological activity and toxicological potential. This will contribute to providing best practice guidelines for using these species when they are cultivated in urban environments for medicinal purposes.

Statements and Declarations

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Competing interests

All authors declare that there are no actual or potential conflicts of interest, including financial, personal, or other relationships with other people or organizations.

Author contributions

All authors contributed to the conception and design of the study. Laís Bezerra prepared the material and collected and analyzed the data. Marcelino José dos Anjos and Ramon Santos conducted the chemical soil analysis. The first draft of the manuscript was written by Laís Bezerra, and all authors contributed to subsequent versions. All authors participated in the data analysis and discussion of the results.

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