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Biomonitoring of air pollution: a dichotomous key for lichen species identification

Biomonitoramento da poluição do ar: uma chave dicotômica para identificação de espécies de líquens

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ABSTRACT

Biomonitoring has been seen as complementary analysis to physicochemical methods and as a low-cost alternative, mainly for regions lacking air pollution control programs. Despite being the most widely used bioindicator for this type of pollution, lichens are not easily identified and this methodology is restricted to groups of expert researchers. Accordingly, the aim of the present study was to analyze lichen diversity in urban and industrial areas, in order to elaborate a dichotomous key to identify the listed species, based on easily visualized features. Lichens with leaf stalks belonging to family Parmeliaceae and genus Parmotrema were the most abundant, which are known for their resistance to air pollution. The study sites generally presented low lichen diversity, which may also have been influenced by the degree of vegetation around them. This result reinforces the importance of green areas for mitigating air pollution in urban environment. The dichotomous key elaborated for the lichen species found aims to support research, environmental education and management activities, and may expand the use of lichens as air-quality bioindicators.

Keywords: Atmospheric pollution; Bioindicators; Lichen identification

RESUMO

O biomonitoramento tem sido considerado como uma análise complementar aos métodos físico-químicos e como uma alternativa de baixo custo, principalmente para regiões carentes de programas de controle da poluição do ar. Apesar de ser o bioindicador mais utilizado para esse tipo de poluição, os líquens não são facilmente identificados, ficando essa metodologia restrita a grupos de pesquisadores especializados. Nesse sentido, o objetivo do presente estudo foi analisar a diversidade de líquens em áreas urbanas e industriais, a fim de elaborar uma chave dicotômica para identificar as espécies listadas, com características de fácil visualização. Líquens foliosos pertencentes à família Parmeliaceae e ao gênero Parmotrema foram os mais abundantes, os quais são conhecidos por sua resistência à poluição atmosférica. Os locais do estudo apresentaram, em geral, baixa diversidade de líquens, a qual pode ter sido influenciada também pela grau de vegetação no entorno dos pontos. Esse resultado reforça a importância de áreas verdes para mitigar a poluição do ar em ambiente urbano. A chave dicotômica elaborada para as espécies de líquens encontradas visa apoiar a pesquisa, atividades de educação ambiental e de manejo, podendo ampliar o uso de líquens como bioindicadores da qualidade do ar.

Palavras-chave: Poluição atmosférica; Bioindicadores; Identificação de líquens

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1 INTRODUCTION

Bioindicators have been used as complementary analysis and low-cost alternative to physicochemical methods, mainly in regions lacking air pollution monitoring and control programs (YATAWARA; DAYANANDA, 2019). Biomonitoring aims at analyzing organisms' quantitative and qualitative responses to environmental changes. This study followed a defined spatial and temporal scale to help identifying reference and critical air quality areas in a given region (PARVIAINEN et al., 2019).

Lichens stand out among air quality bioindicators for their wide geographical distribution, even in areas subjected to severe climatic conditions, and for their sensitivity to air pollution. Air purity is one of the main factors assuring lichen survival, since these organisms have hygroscopic eating habit, as well as accumulate and fix dispersed elements in the air. Lichens do not have stomata and cuticles like vegetables do; therefore, gases and some air components can be absorbed and reach photobiont component cells. The absence of stomata and cuticles highlights lichens' inability to excrete toxic substances; they tend to accumulate absorbed metal components. Moreover, these organisms absorb water straight from the atmosphere, which makes them more exposed to the action of pollutants (MARCELLI, 2006).

Most air quality biomonitoring studies analyze lichen diversity as environmental change indicator (KÄFFER et al., 2011; RAIMUNDO COSTA; MINEO, 2013; CUNHA; MARCELLI; PEREIRA, 2015; YATAWARA; DAYANANDA, 2019). Despite being the most widely used organisms for pollution control, lichens are not easily identified, since this methodology is restricted to groups of expert researchers. Lichenology is little studied in Brazil, and it accounts for the technical difficulty to identify different lichen species and to expand their use as bioindicators (MARCELLI, 2006).

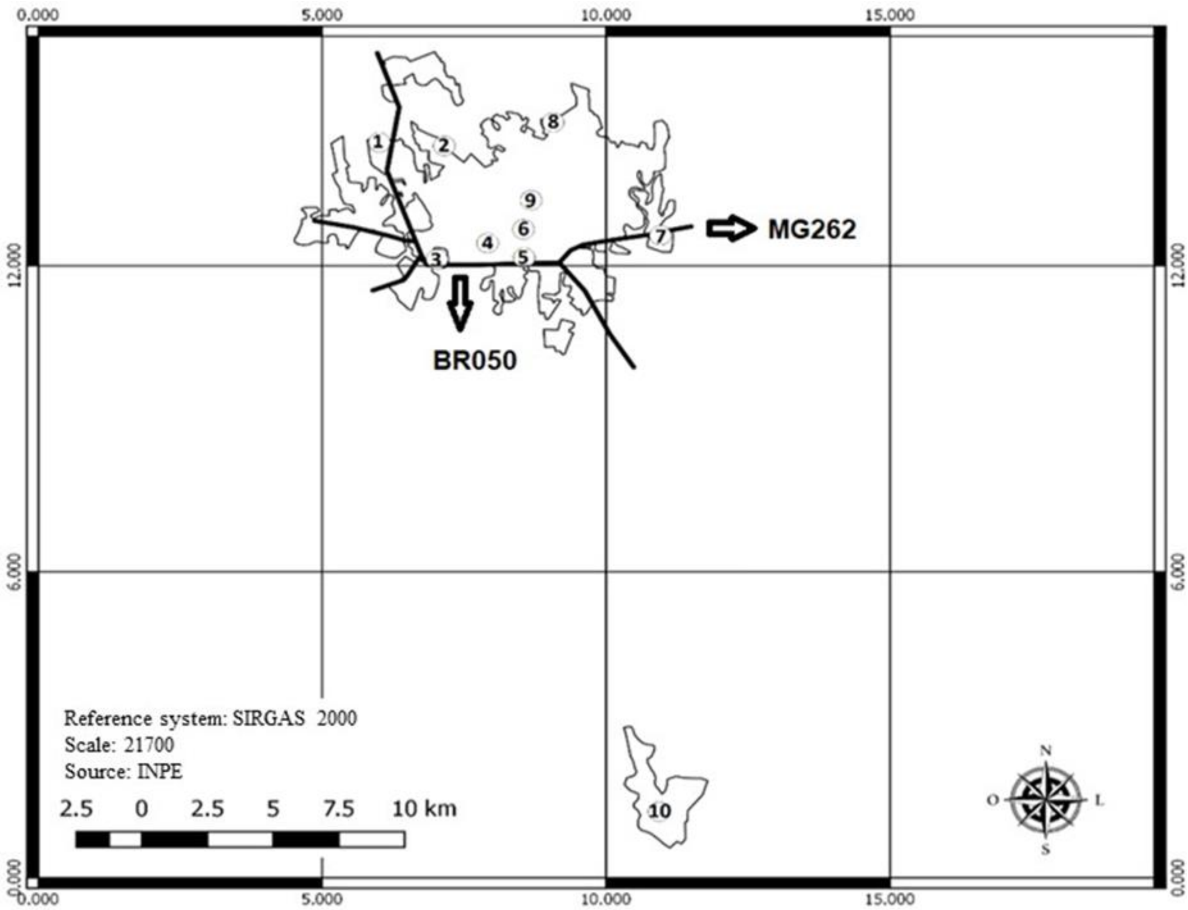
Accordingly, the present study proposed an analysis of lichen diversity in urban and industrial areas, and elaborated a dichotomous key to identify the listed species in order to contribute to air quality biomonitoring practices.

2 MATERIAL AND METHODS

The study was carried out in Uberaba County, Triângulo Mineiro region, Minas Gerais State, which covers a territory of 4,523,957 km² and houses an estimated population of 334,000 inhabitants - over 90% of whom live in urban areas (IBGE, 2019). Air pollution monitoring and control is not carried out by competent state agencies in the region, although it is a mid-sized county with growing car fleet.

In total, 9 study sites were set for the survey on lichen species in urban and one was set in industrial area. Sites 1, 3, 4, 5 and 7 were located 50 to 300 meters away from highways. Two sites (6 and 9) were located in downtown Uberaba and two sites (2 and 8) were placed farther away from possible pollution sources, in residential neighborhoods. Study site 10 was the only one located in an industrial area, 20 km from Uberaba City.

Figure 1- Location of the study points in Uberaba-MG



Source: authors

2.1 Collection and identification of lichen samples

Samples of lichens presenting different sizes - yet fixed at the bark of phorophyte trees - were collected in an area measuring radius of approximately 30 m around the study points. Samples located between the basis of phorophyte trees and the maximum height of 2 m on their bark were taken into consideration for lichen identification.

The collected material was packed in labeled envelopes: collection date and location. Lichen identification was carried out with the aid of specific image catalogs imagens (MARCELLI, 2006; FLEIG et al., 2008; APTROOT; SPARRIUS, 2018).

Carbon and Potassium tests were performed to identify some species. The carbon test consisted in using sodium hypochlorite as indicator of acid lichen formation. A small amount of C solution was applied on the stem cortex of each collected lichen and it stained the treated area. The potassium test consisted in using potassium hydroxide to identify the presence of lichen acids, since such solution changes stalk coloration where it is applied.

2.2 Dichotomous identification key

The elaborated dichotomous key was based on identifying lichens sampled at specific or gender level. Taxa were divided into three groups according to lichen type (foliose, crustose and fruticose). In addition to (positive or negative) reactions observed in the C and K tests (positive or negative), other features, such as the presence and color of ascomata, cilia, isidia/soridium, pseudocyphellae, macules, lower cortex, stalk size and staining, were identified in a stereoscopic microscope (40x).

2.3 Data Analysis

Lichen diversity between study sites were compared through Sörensen index similarity analysis, which ranges from 0 (similarity-free) to 1 (maximum similarity) (Equation 1). This index qualitatively compares species equivalence between successive samples removed at spatial intervals or along an environmental gradient. Sörensen index similarity analysis formula is given by:

$$Is = \frac{2c}{(a+b)} \quad (1)$$

Onde:

Is = Sörensen index similarity;

a = number of species occurring at site A;

b = number of species occurring at site B;

c = number of species common to both sites compared.

3 RESULTS AND DISCUSSION

In total, 28 lichen species belonging to 15 genera and 11 families were identified (Table 1). Foliose lichens were the most abundant species (82.1%), which was followed by crostose (14.3%) and fruticose (3.6%). The lichens type was the first entry of the dichotomous key, which is shown in Table 3.

Table 1 - Lichen diversity observed at different study points in Uberaba-MG

TAXON	Study points										TYPE
	1	2	3	4	5	6	7	8	9	10	
ARTHONIACEAE Rchb. (1941)											
Cryptothecia candida (Kremp) R. Sant.	X	X		X	X	X		X			Crostose
BIATORACEAE A. Massal. ex Stiz. (1862)											
Bacidia sp. De Not.	X	X	X	X		X	X			X	Crostose
CALICIACEAE Chevall.											
Dirinaria applanata (Fée) D.D. Awasthi	X	X			X						Foliose
Dirinaria melanocarpa C.W. Dodge	X										Foliose
Dirinaria picta (Sw.) Clem. & Shear					X				X		Foliose
CANDELARIACEAE Hakul											
Candelaria concolor (Dicks.) Stein	X	X	X	X	X		X				Foliose
Candelaria fibrosa (Fr.) Müll.Arg.				X			X		X		Foliose
COCCOCARPIACEAE Henssen											
Coccocarpia sp. Pers. (1827)					X						Foliose
LECANORACEAE Körb. (1855)											
Lecanora sp. 1 Ach.	X		X	X	X	X		X			Foliose
Lecanora sp. 2 Ach.		X	X		X			X			Foliose
Lecanora sp. 3 Ach.					X		X				Foliose
Lecanora sp. 4 Ach.								X			Foliose

Pyrrhospora russula Ach.	X										Crostose
LETROUITIACEAE Bellem. & Hafellner (1982)											
Letrouitia transgressa (Malme) Hafellner & Bellem.					X						Crostose
PARMELIACEAE Zenker (1827)											
Canoparmelia sp. (Tuck.) Elix & Hale			X			X					Foliose
Canoparmelia texana (Tuck.) Elix & Hale						X	X		X	X	Foliose
Parmelia sp. 1 (L.)					X	X	X				Foliose
Parmelia sp. 2 (L.)					X						Foliose
Parmelinopsis minarum (Vain.) Elix & Hale					X	X	X		X	X	Foliose
Parmelinopsis sp. Elix & Hale		X					X		X		Foliose
Parmotrema aurantiacoparvum Sipman	X										Foliose
Parmotrema mesotropum (Müll. Arg.) Hale	X										Foliose
Parmotrema presorediosum (Nyl.) Hale		X				X					Foliose
Parmotrema sancti-angeli (Lynge) Hale				X	X		X	X			Foliose
Parmotrema tinctorum (Despr. ex Nyl.) Hale	X	X	X	X	X	X		X	X		Foliose
PERTUSARIACEAE Körb. ex Körb. (1855)											
Pertusaria sp DC.	X					X					Crostose
PHYSIACEAE Zahlbr. (1898)											
Physcia aipolia (Humboldt) Fürnrohr	X										Foliose
USNEACEAE L.											
Usnea sp. Dill. ex. Adans. (1763)	X					X					Fruticose
SPECIES RICHNESS	13	8	6	7	14	11	9	6	6	3	

Source: authors

Table 2 - Sörensen similarity index between the lichen diversity of different study points in Uberaba – MG

Study points	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**
P1	1.00									
P2	0.48	1.00								
P3	0.43	0.63	1.00							
P4	0.43	0.50	0.57	1.00						
P5	0.38	0.48	0.43	0.43	1.00					
P6	0.43	0.48	0.53	0.42	0.36	1.00				
P7	0.16	0.44	0.25	0.50	0.40	0.38	1.00			
P8	0.35	0.50	0.57	0.57	0.52	0.42	0.13	1.00		
P9	0.17	0.38	0.29	0.29	0.35	0.42	0.50	0.29	1.00	
P10	0.11	0.17	0.20	0.20	0.11	0.40	0.50	0.00	0.40	1.00

Source: authors

Leafy lichens are more common in urban areas; Käffer et al. (2011) found positive correlations between vehicle flow and the richness of these species richness, which confirms their high resistance to air pollution.

Genus Usnea was the only fruticose genus found at study sites 1 and 6. Site 1 was located near the highway and site 6 was placed in the central region. Despite the location, air pollution did not limit the presence of this sensitive genus. Both study sites had green areas in their surrounding areas. According to Käffer et al. (2011), there is positive correlation between the ecological status of an area and the presence of fruticose lichen species; therefore, the better the environmental conditions in the area, the greater the richness, coverage and diversity of lichens, and the variety of fruticose lichens.

Family Parmeliaceae (39.3%) was the one presenting the largest number of species; it was followed by Lecanoraceae (17.9%) and Caliciaceace (10.7%). Genus *Parmotrema* was the most common one, it represented 17.9% of the sampled species.

Family Parmeliaceae hosts the species presenting the greatest dominance and richness in Brazil in high illumination zones. Genus *Parmotrema* is the most frequent one in lichen communities given the greater diversity of its species; therefore, it is the most assessed genus by Brazilian lichenologists (SPIELMANN; MARCELLI, 2008).

Parmotrema tinctorum was the species recording the highest frequency in the study sites; it was found in 80% of the analyzed sites. This species was followed by *Bacidia* sp. and *Chrysothrix* sp., which were found in 70% of the assessed sites.

Genus *Bacidia* can be found from the Arctic to tropical regions and genus *Chrysothrix*, also called gold dust lichen - due to its bright yellow to greenish yellow color -, is composed of American lichen species. Species belonging to both genera use tree bark and, in some cases, rocks or soil as basis or support (CONSORTIUM, 2018; GOLD, 2018).

Parmotrema tinctorum is an urban species resistant to air pollutants; therefore, it is a good air-pollution indicator (MARTINS; KÄFFER; LEMOS, 2008). Active biomonitoring studies have been using such indicator to analyze the accumulation of potentially toxic metals (BOONPENG et al., 2017).

Study site 10 recorded the lowest lichen diversity; only three species (*Bacidia* sp., *Canoparmelia texana* and *Parmelinopsis minarum*) were identified in this site (Table 1). The explanation to pollution in this site lies on the fact that it was set in an area subjected to activities related to chemical, fertilizer, fuel distribution and carrier industries, as well as to high vehicle flow, mainly trucks, that, in their turn, generate burning and compounds harmful to lichen survival (RAIMUNDO COSTA; MINEO, 2013).

Species *Canoparmelia texana* belongs to family Parmeliaceae and is one of the most widely distributed species in open spaces and in urban areas throughout Brazil. It stands out among the three species found in site 10; therefore, it has been extensively assessed for air quality biomonitoring (SAIKI et al., 2013). Overall, *C. texana* occupies large tree trunks and is commonly seen in parks, squares and university campuses; its important features, such as tolerance to high pollution levels, allow its use as bioaccumulator of chemical elements (FUGA et al., 2008).

Results of the similarity analysis applied to the study sites are shown in Table 3. There was low similarity between species found in site 10, which is located in an industrial area, when they were compared to lichens found in other areas (23.2%, on average). Lichens found in sites 6, 7 and 9 recorded the greatest similarities with the ones in site 10, because the most resistant species found in site 10 were also found in these sites.

Table 3 - Dichotomous key for lichen species identification

1a. Foliose lichen.	.2
1b. Crostose lichen..	18
1c. Fruticose lichen..	*Usnea* sp.
2a. With ascoma....	3
2b. Without ascoma.....	10
3a. C test - positive...	4
3b. C test - negative..	6
4a. K test - positive.	*Parmotrema tinctorum*
4b. K test - negative.	5
5a. With cilium	*Parmorrema mesotropum*
5b. Without cilium.	*Parmotrema presorediosum*
6a. With macula.	*Parmelia* sp. 2
6b. Without macula..	7
7a. Yellowish stalk.	*Candelaria fibrosa*
7b. Gray/greenish stalk..	8
8a. With isidia/soredia..	*Dirinaria applanate*
8b. Without isidia/soredia.	9
9a. Ascoma with black interior..	.*Lecanora* sp. 2
9b. Ascoma with white interior.	*Physcia aipolia*
10a. C test - positive..	11

10b. C test - negative..	14
11a. With cilium…	12
11b. Without cilium/yellowish stalk..	*Candelaria concolor*
12a. With isidia/soredia..	13
12b. Without isidia/soredia.	*Parmelinopsis* sp.
13a. Stalk with lobes.	.*Parmotrema sancti-angeli*
13b. Stalk with lobules.	*Parmelinopsis minarum*
14a. With cilium..	*Parmotrema aurantiacoparvum*
14b. Without cilium.	15
15a. With isidia/soredia.	16
15b. Without isidia/soredia..	17
16a. Sleasy stalk..	*Dirinaria picta*
16b. Stalk with lobes..	*Canoparmelia texana*
16c. Stalk corticated on both surfaces, with rhizines on the side..	*Parmelia* sp. 1
17a. With pseudocyphella..	*Canoparmelia* sp.
17b. Without pseudocyphella.	*Coccocarpia* sp.
18a. With ascoma..	19
18b. Without ascoma..	20
19a. Lecideine ascoma, with orange margin	*Pyrrhospora russula*
19b. Lecideine ascoma, with yellowish margin	*Letrouitia transgressa*
19c. Lecanorine ascoma..	21
20a. Sleasy stalk.	*Pertusaria* sp.
20b. Farinose stalk	*Bacidia* sp.
21a. C test - positeve.	*Lecanora* sp. 1
21b. C test - negative.	22
22a. With pseudocyphella	*Dirinaria melanocarpa*
22b. Without pseudocyphella…	23
23a. With lower cortex..	24
23b. Without lower cortex	*Cryptothecia candida*
24a. Light gray stalk	*Lecanora* sp. 4
24b. Dark greenish gray stalk.	*Lecanora* sp. 3

Source: authors

Study sites 1 and 5 were the ones recording the highest lichen richness (13 and 14 species, respectively) (Table 1). These sites were located very close to the highway, where one finds high air pollution levels, but they had squares surrounded by several trees. Despite the great diversity of lichens, species found in these sites, such as *Dirinaria picta*, *Canoparmelia texana*, *Parmotrema tinctorum*, *Physcia aipolia,* were typical of urban areas and tolerant to air pollution (MARTINS; KÄFFER; LEMOS, 2008; CUNHA; MARCELLI; PEREIRA, 2015).

A study conducted in Recife-PE compared a square to an avenue and showed greater lichen coverage in the square, although it was located in the downtown area and surrounded by high flow of vehicles (SANTOS et al., 2015). Green areas improve the quality of life and of the environment by exercising their ecological, aesthetic and leisure functions; therefore, it is essential to take into consideration their presence in urban planning. Vegetation tends to make the atmosphere cleaner by changing wind speed and direction, modifying pollutant dispersion and increasing particulate deposition (JANHALL, 2015; KLINGBERG et al., 2017).

4 CONCLUSIONS

Results have shown that foliose lichens belonging to family Parmeliaceae and genus *Parmotrema* presented the highest abundance among the observed species, since they are common in urban areas given their resistance to atmospheric pollution. The site established in the industrial area presented the lowest lichen richness; however, in addition to air pollution level, lichen diversity may have been influenced by the presence of surrounding trees. Moreover, results in the present study corroborate data in the literature, which allowed identifying urban green areas as a possible way to mitigate atmospheric pollution.

The dichotomous key elaborated for lichen species was based on easily visualized characteristics. This tool is expected to support future research, environmental education and management activities in the environment and health knowledge field by strengthening lichens' use for air quality biomonitoring.

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