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Short Communication

The use of *Parmotrema tinctorum* (Parmeliaceae) as a bioindicator of air pollution

William Raimundo-Costa[1,2,3], *Deusmaque Carneiro Ferreira*[2,4], *Ana Carolina Borella Marfil Anhê*[2,5] & *Ana Paula Milla dos Santos Senhuk*[2,6,7]

Abstract
Air quality monitoring by automatic stations, although efficient, does not allow evaluating the effects of pollution on living organisms and communities. Thus, the aim of the present study was to use lichens of the *Parmotrema tinctorum* species in active air quality biomonitoring. We used a new methodology of chlorosis area analyses in QGis software, as low-cost and complementary tool to physicochemical methods. Samples of the aforementioned species were exposed to atmospheric pollution for 30 consecutive days in the dry and rainy seasons, in urban and industrial regions. The chlorosis rate (34% of the lichen thalli, on average) and the accumulation of sulfur (1.1 $g.kg^{-1}$, on average) were higher in the samples of lichens exposed in the industrial region, in the dry season. There was a moderate-to-high positive correlation between chlorosis rate and lichen content of nitrogen, sulfur, iron and zinc, in the dry season, mainly with sulfur ($r = 0.71$). The results confirmed the sensitive of *P. tinctorum* to atmospheric pollution, even after a short exposure time. Such new active biomonitoring methodology (chlorosis analysis in the QGis) can be used in future studies of air quality assessment by environmental and health surveillance managers.
Key words: active biomonitoring, air quality, lichen.

Resumo
O monitoramento da qualidade do ar por estações automáticas, apesar de eficiente, não permite avaliar os efeitos da poluição sobre organismos e comunidades. Assim, o objetivo do presente estudo foi usar liquens da espécie *Parmotrema tinctorum* em biomonitoramento ativo da qualidade do ar, baseado em uma nova metolologia de análise de clorose por meio do software Qgis, como uma ferramenta de baixo custo e complementar aos métodos físico-químicos. Amostras dessa espécie foram expostas à poluentes do ar por 30 dias consecutivos, nas estações seca e chuvosa, em regiões urbanas e industriais. A taxa de clorose (34% do talo dos liquens, em média) e o acúmulo de enxofre (1,1 $g.kg^{-1}$, em média) foram maiores em amostras expostas na região industrial, na estação seca. Houve correlação positiva moderada a alta entre a taxa de clorose e o acúmulo de nitrogênio, enxofre, ferro e zinco, no período seco, principalmente com exnofre ($r = 0{,}71$). Os resultados confirmaram a sensibilidade de *P. tinctorum* à poluição atmosférica, mesmo após um curto período de exposição. Essa nova metodologia de biomonitoramento ativo (análise de clorose por meio do QGis) pode ser usada em estudos futuros sobre avaliação da qualidade do ar por gestores de vigilância ambiental e de saúde.
Palavras-chave: biomonitoramento ativo, qualidade do ar, líquen.

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[1] Municipal Health Secretary, Dep. Environmental Health Surveillance, Uberaba City Hall, Uberaba, MG, Brazil.
[2] Federal University of Triangulo Mineiro, Postgraduate Prog. Environmental Science and Technology, Uberaba, MG, Brazil.
[3] ORCID: <https://orcid.org/0000-0001-8971-6766>. [4] ORCID: <https://orcid.org/0000-0001-9338-0863>.
[5] ORCID: >https://orcid.org/0000-0002-6970-2479>. [6] ORCID: <https://orcid.org/0000-0002-6004-5513>.
[7] Author for correspondence: ana.senhuk@uftm.edu.br

Air quality is often assessed through physicochemical means in automatic monitoring stations, which measure pollutants at local scale. These methods are efficient, but do not allow evaluating the systemic effects of pollution on living organisms and communities. Biological indicators can show these impacts with results in the short-, mid- and long-term, not necessarily using high-cost equipments. Although biomonitoring should not replace physicochemical methods, it can provide complementary information about biological damages caused by pollutants, mainly about the cumulative ones (Ellenberg *et al*. 1991; Klumpp 2001; Saiki *et al.* 2014; Lucheta *et al.* 2019).

Lichens stand out as one of the most efficient living organisms used as bioindicators of air pollution (Nimis *et al.* 1990). This happens due several characteristics such as: a) lack of protective layers such as cuticle or serous layers, as seen in phanerogams; b) wide geographical distribution; c) lack of excretion structure, which allows compounds absorbed throughout lichens' life to be retained in their thallus (Raven *et al.* 2014).

Certain elements that are incorporated in the lichens can be found in different parts of the thallus, from its surface, linked to cell membranes or in connections with intracellular substances, such as proteins. The accumulation process of these elements in the lichen thalli occurs in different ways, which can be due to simple deposition on the surface of the cortex, by accumulation between the fungi hyphae, by passive transport in response to differences in osmotic and electrical potential, or also by active transport, when membrane connections occur (Bargagli & Mikhailova 2002; Mazitelli *et al*. 2006).

Passive biomonitoring uses organisms that already exist in a given area, while active biomonitoring is done with the exposure of organisms in the area to be evaluated for a defined time and under controlled conditions (Klumpp 2001). Most studies passively assess lichen diversity based on the association between low species richness and increased pollution rates in the investigated. These studies are of paramount importance because they help better understand lichen communities' response to prolonged exposure to air pollutants. However, adequate sampling efforts and prior knowledge about the subject are required to enable lichen species identification environments (Raimundo-Costa & Mineo 2013; Luchetta *et al.* 2018; Luchetta *et al.* 2019; Loppi 2019). On the other hand, active biomonitoring has been mainly based on the analysis of potentially toxic metal accumulations in lichen thalli and morphophysiological damages (Käffer *et al.* 2012; Saiki *et al.* 2014; Koch *et al.* 2018; Port *et al.* 2018).

In this context, this study aimed to use *Parmotrema tinctorum* (Despr. *ex* Nyl.) Hale (Parmeliacea) in active air quality biomonitoring, based on a new methodology of chlorosis area analyses in QGis software, comparing to metals content in lichen thalli.

This species was selected because it has wide geographical distribution, it is easily recognizable in the field and because it is sensitive to atmospheric pollution (Benatti & Marcelli 2009; Käffer *et al.* 2012; Koch *et al.* 2018; Port *et al.* 2018). Its foliose thallus ranges from silver to greenish gray, or to olive green when it is wet; it presents full or sub-crenated margins, as well as smooth upper cortex, which may be rough or cracked and present ridges on the ridges or simple/granular fissures (Benatti & Marcelli 2009). In addition, *P. tinctorum* positively responds to the "C" test (contact with sodium hypochlorite), since it shows orange color in contact with the reagent; as well as to the "K" test, since it shows reddish color in contact with potassium hydroxide (Fleig & Grüninger 2008; Benatti & Marcelli 2009).

The study was conducted in Uberaba Municipality, Triângulo Mineiro region, Minas Gerais state, Brazil, whose estimated population comprises 334 thousand inhabitants (IBGE 2020). Uberaba has the 4th largest vehicle fleet in Minas Gerais state (Denatran 2020); it is crossed by two important highways and has four industrial districts. However, the region lacks air quality monitoring and control program, which is only available at the state's capital.

Parmotrema tinctorum samples were collected in a Conservation Unit located in a rural area (30 km North of the urban center; 19°32'34.10"S, 47°53'38.22"W) which is less exposed to atmospheric pollution. They were collected with part of the substrate (tree bark) and remained in the laboratory for one week for acclimation and physiological homogenization purposes (Martins-Mazzitelli *et al.* 2006). During this period, lichen samples were drawn and measured in the QGis software version 2.18, in order to be compared to macroscopically changed areas of each lichen thallus, after the exposure period. Thus, it was possible calculating the total lichen thallus area and the affected area rate (chlorosis and/or necrosis).

This software, usually used for geographical analyses and making maps, was quite efficient since it has efficient tools for checking previously inserted areas using raster or vector files (produced manually). In the present study, scale measures in meters were established in the program environment, thus being able to calculate the area values for each lichen thalli, which were inserted as vector-type files, drawn from photographs in scale 1 to 1 of the lichen stalks themselves. No plug-ins were used, only layers of polygons and the area function in QGis' own calculator.

Lichen samples were exposed to atmospheric pollution for 30 consecutive days in the dry (August to September) and rainy (December to January) seasons. Lichen thalli were transplanted from the laboratory (after the period for acclimation) to each exposure points, which comprised three samples per point, set in trees at 2 m above the ground. About four thalli was exposed in each sample, with 12–15 cm in diameter each thallus, approximately. The samples were transported in sterile vials in order to avoid contamination and loss of material that could compromise the results.

Lichen exposure points (in supplementary data <https://doi.org/10.6084/m9.figshare.12797468.v1>) were distributed as follows: three points in urban areas (A, B and C), with air pollution specially by vehicle emissions, and one point in industrial area (D) presenting chemical, fertilizer and fuel distribution industries (19°58'9.25"S, 47°53'22.46"W). Points A (19°44'3.68"S, 47°59'13.45"W) and B (19°46'39.33"S, 47°56'1.09"W) were in residential areas, while point C was downtown (19°45'5.83"S, 47°55'55.74"W), 40 meters away from an important avenue that presented intense traffic on a daily basis. This point was a typical commercial area, which hosted a few homes and many vertical condominiums that hindered airborne pollutant spreading.

Control group samples were kept in sterile vials in the laboratory during the exposure period.

The thalli from the exposure points and from the control group were homogenized to obtain 2 g per sample for acid digestion and further analysis of some chemical element concentration related to air pollution (nitrogen (N), sulfur (S), copper (Cu), iron (Fe) and zinc (Zn)). The analyzes were performed in a private laboratory, by atomic emission spectrometer (Agilent MP AES 4100), in order to validate the chlorosis analysis.

Chlorosis rate and pollutant concentration data were compared between exposure points based on analysis of variance (one-way ANOVA) and Tukey's test for parametric data, or on Kruskal-Wallis and Dunn tests for non-parametric ($p < 0.05$). The data were also compared between the dry and rainy seasons by T test or Mann-Whitney ($p < 0.05$), in the BioEstat 5.3 software.

Lichen thalli at all exposure points showed pink shade chlorosis, which is characteristic of exposure to S (Ellenberg *et al*. 1991). Some toxic air elements have affinity to chloroplasts, where the chlorophyll can be degraded to pheophytin, leading to the emergence of chlorosis and, later, of necrosis areas (Ellenberg *et al*. 1991; Bargagli & Mikhailova 2002). Photobiont vitality in samples of *P. tinctorum* has decreased in urban and industrial areas; this morphological damage is a sensitive variable in active biomonitoring, considered as an initial response to the air pollutants exposure (Port *et al.* 2018).

Lichens recorded higher chlorosis rates during the dry season, as seen in the dark areas of Figure 1 (supplementary data <https://doi.org/10.6084/m9.figshare.12797468.v1>). Significant difference in chlorosis rates was observed between exposure points, in the dry season; the highest value was recorded for the industrial region (34% of lichen thalli, on average) (Tab. 1). Although no significant differences were observed between exposure points in the rainy season, the chlorosis rate tended to present higher values in the industrial (D) and central (C) regions.

Pearson's coefficient showed moderate-to-high positive correlation between chlorosis rate in the lichen thalli and N, S, Fe and Zn concentrations (Tab. 2). The highest correlation was recorded between chlorosis rate and S concentration ($r = 0.71$).

Based on results of chemical element accumulation in lichen thalli, spatial variation was observed only for the concentrations of S, during the dry season; it was higher in the industrial region (D), with 1.1 $g.kg^{-1}$ on average (Tab. 1). The concentrations of N, Fe and Zn tended to be higher in the industrial region (D), and Cu higher in the central region (C), although there was no significant difference.

Some averages showed a very high standard deviation; so it is suggested to increase the number of samples and, if possible, to leave them exposed for a longer time, for future studies, in order to increase the accumulation of chemical elements related to air pollution. Studies carried out with *P. tinctorum* in active biomonitoring adopted a longer

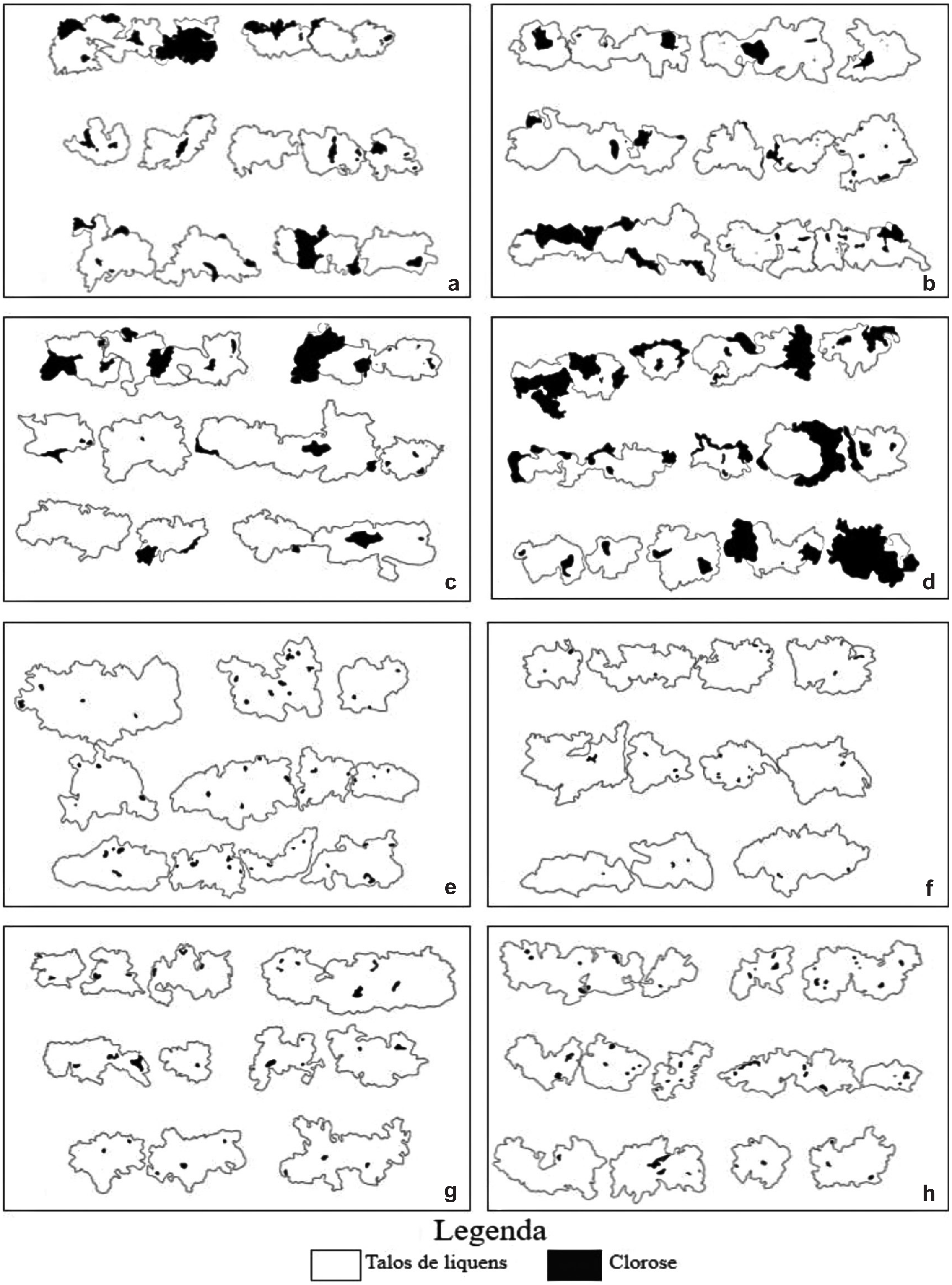

Figure 1 – a-h. Chlorosis area verified in lichen thalli – a-d. samples exposed in the dry season at sampling points A, B, C and D, respectively; e-h. samples exposed during the rainy season at sampling points A, B, C and D, respectively (in supplementary data).

Table 1 – Mean of chlorosis (%) and concentration of polluting elements in samples of lichens from different points, in the dry and rainy season.

Season / local	N ($g.kg^{-1}$)	S ($g.kg^{-1}$)	Cu ($mg.kg^{-1}$)	Fe ($g.kg^{-1}$)	Zn ($mg.kg^{-1}$)	Chlorosis (%)
Dry						
Control	10.6 ± 2.1	0.9 ± 0.1[ab]*	8.1 ± 0.8	2.7 ± 0.4*	17.6 ± 2	0
A	8.5 ± 4.3	0.7± 0.1[a]	6.5 ± 1.3	2.3 ± 0.6*	16.4 ± 0.7	11.7 ± 11.4[ab]
B	10.8 ± 0.8	0.9± 0[ab]*	7.8 ± 0.9	2.6 ± 0.2*	16.9 ± 0.8	9.4 ± 5.8[a]
C	10.7 ± 1.2	0.9± 0.2[ab]*	12.6 ± 5.7	3.3 ± 0.9*	18.6 ± 3.2	14.6 ±10.3[ab]
D	12.2 ± 1.2*	1.1 ± 0[b]*	8.1 ± 2.2	3.5 ± 0.06*	18.6 ± 1.8	33.9 ± 8.1[b]*
Rainy						
Control	10.1 ± 1.7	0.6 ± 0.1	6.2 ± 3.4	0.7 ± 0.1	18 ± 2.4	0
A	10.1 ± 0.3	0.7 ± 0.2	5.5 ± 0.6	0.7 ± 0.3	16.7 ± 3	1.5 ± 0.5
B	9.6 ± 0.8	0.8 ± 0	6.3 ± 1.4	0.8 ± 0.03	20.2 ± 4.3	0.6 ± 0.2
C	10.9 ± 1.2	0.6 ± 0	7.6 ± 1.7	0.8 ± 0.03	18.8 ± 4.2	1.7 ± 0.9
D	9.5 ± 1	0.7 ± 0	5.8 ± 1.3	0.9 ± 0.04	17.7 ± 4	1.9 ± 0.5

Small letters indicate significant difference between study points, in each season, by Anova/Tukey or Kruskal-Wallis/Dunn tests ($p < 0.05$). Asterisk (*) indicates the highest values comparing the dry and rainy seasons, in each study point, by T test ($p < 0.05$).

exposure time (7 months) and recorded higher concentrations of S and metals, as well as more significant morphophysiological damages (Käffer *et al.* 2012; Koch *et al.* 2018).

The industrial region comprised chemical and fertilizer industries that use S and N in their production processes, stood out. The intense traffic in the central and industrial regions is also responsible for releasing air pollutants deriving from fuel combustion (Braun *et al*. 2004; Lopes *et al*. 2018). Cu, Fe and Zn found in the atmosphere may be of vehicular origin, since one of the emission sources of these elements refers to the wearing of automotive parts, tires and brakes, whose particulate matter deposited on different surfaces is continuously resuspended in the environment (Qi *et al*. 2016). These elements are also found in the composition of motor oils, which have potential to increase their concentration in the environment (Silveira *et al.* 2010).

The concentrations of S, Cu and Fe were higher in the dry season, regardless of the region (Tab. 1). The seasonal difference may be associated with lower temperature and rainfall records in the region, which are common in winter and, consequently, with lower pollutant dispersion. On the other hand, the concentration of chemical elements in the atmosphere tends to be lower in the

Table 2 – Pearson correlation matrix between variables collected in the dry period, in the studied points.

	N	**S**	**Cu**	**Fe**	**Zn**	**Chlorosis (%)**
N	1	---	---	---	---	---
S	0.99	1	---	---	---	---
Cu	0.31	0.21	1	---	---	---
Fe	0.84	0.85	0.6	1	---	---
Zn	0.79	0.78	0.71	0.99	1	---
Chlorosis (%)	0.59	0.71	0.22	0.62	0.51	1

N = Nitrogen; S = Sulfur; Cu = Copper; Fe = Iron; Zn = Zinc.

rainy season, since they are removed from the air and incorporated in water particles (Baird & Cann 2011; Ouyang *et al*. 2015).

The results confirmed the sensitive of *P. tinctorum* to atmospheric pollution, even after a short exposure time. It could increase the accumulation of chemical elements related to such pollution and find significant differences between the points studied, with a longer time of exposure. On the other hand, as the morphological effects, with the gradual loss of the photobiont component, are the first responses to air polluted, the analysis of chlorosis using the QGis software proposed here proved to be effective in providing fast results.

As a low-cost tool, complementary to the physical-chemical methods of monitoring air pollutants, this new methodology can be used to improve researches on active biomonitoring and air quality assessment by environmental and health surveillance managers.

Supplementary data: <https://doi.org/10.6084/m9.figshare.12797468.v1>.

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